ZIDOVUDINE (AZT)

1. Exposure Data

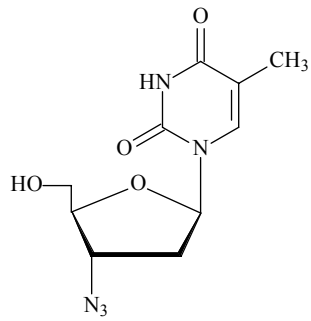
1.1 Chemical and physical data

Zidovudine is an analogue of thymidine in which the 3-hydroxyl group is replaced by an azido group.

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 30516-87-1
Chem. Abstr. Name: 3′-Azido-3′-deoxythymidine
IUPAC Systematic Name: 3′-Azido-3′-deoxythymidine
Synonyms: 1-(3-Azido-2,3-dideoxy-β-D-ribofuranosyl)-5-methylpyrimidine-2,4-(1*H*,3*H*)-dione; azidothymidine; 3′-azidothymidine; AZT; 3′-deoxy-3′-azidothymidine; ZDV
[Note: The abbreviation AZT is also used for another drug, azathioprine (Royal Pharmaceutical Society of Great Britain, 1999).]

1.1.2 *Structural and molecular formulae and relative molecular mass*



$C_{10}H_{13}N_5O_4$ Relative molecular mass: 267.25

1.1.3 *Chemical and physical properties of the pure substance*

(*a*) *Description*: White to off-white crystals or needles (Gennaro, 1995; American Hospital Formulary Service, 1997)

(*b*) *Melting-point*: 106–112 °C (from petroleum ether); 120–122 °C (from water) (Budavari, 1996)
(*c*) *Spectroscopy data*: Infrared, ultraviolet, nuclear magnetic resonance (proton) and mass spectral data have been reported (National Cancer Institute, 1989).
(*d*) *Solubility*: Soluble in water (25 mg/mL at 25 °C) and ethanol (67 mg/mL) (Gennaro, 1995; Budavari, 1996)
(*e*) *Optical rotation*: $[\alpha]_D^{25}$, +99° (c = 0.5 in water) (Budavari, 1996)
(*f*) *Dissociation constant*: pK_a, 9.68 (Gennaro, 1995)

1.1.4 *Technical products and impurities*

Zidovudine is available as a 300-mg tablet, a 100- or 250-mg capsule, a 50-mg/5 mL syrup and a 200-mg/20 mL injection solution; it is also available as a tablet in combination with lamivudine. The tablets may also contain macrogol, magnesium stearate, microcrystalline cellulose, povidone, sodium carboxymethyl starch and titanium dioxide. The capsules may also contain corn starch, gelatin, indigo carmine (CI 73015), indigotine, magnesium stearate, microcrystalline cellulose, polysorbate 80, sodium starch glycollate, starch–maize, sulfites, iron oxides and titanium dioxide. The syrup may also contain anhydrous citric acid, flavourings, glycerol, maltitol solution, saccharin sodium, sodium benzoate, sodium hydroxide and sucrose. The injection solution may also contain hydrochloric acid or sodium hydroxide (Gennaro, 1995; Canadian Pharmaceutical Association, 1997; British Medical Association/Royal Pharmaceutical Society of Great Britain, 1998; Editions du Vidal, 1998; LINFO Läkemedelsinformation AB, 1998; Rote Liste Sekretariat, 1998; Thomas, 1998; US Pharmacopeial Convention, 1998a). The oral solution containing 50 mg/5 mL zidovudine is colourless to pale yellow and has a pH of 3–4; the oral solution contains sodium benzoate as a preservative and may contain sodium hydroxide to adjust the pH. The injection for intravenous infusion has a pH of approximately 5.5 and contains no preservatives, but hydrochloric acid and/or sodium hydroxide may be added during manufacture to adjust the pH (American Hospital Formulary Service, 1997).

The following impurities are limited by the requirements of the British and European pharmacopoeias: 1-[(2*R*,5*S*)-5-hydroxymethyl-2,5-dihydro-2-furyl]-5-methylpyrimidine-2,4(1*H*,3*H*)-dione; 1-(3-chloro-2,3-dideoxy-β-D-ribofuranosyl)-5-methylpyrimidine-2,4(1*H*,3*H*)-dione; thymine; and triphenylmethanol (British Pharmacopoeia Commission, 1996; Council of Europe, 1997).

Trade names for zidovudine include Apo-Zidovudine, Azitidin, Azoazol, Azotine, Azovir, AZT Filaxis, Crisazet, Enper, Exovir, Novo-AZT, Retrovir, Virustat, Zidosan, Zidovir, Zidovudina Combino Pharm, Zidovudina Lazar and Zidovudine Asofarma (Swiss Pharmaceutical Society, 1999).

1.1.5 *Analysis*

Several international pharmacopoeias specify infrared absorption spectrophotometry with comparison to standards and liquid chromatography as the methods for identifying zidovudine; liquid chromatography is used to assay its purity. In pharmaceutical preparations, zidovudine is identified by ultraviolet absorption spectrophotometry, thin-layer chromatography and liquid chromatography; liquid chromatography is used to assay for zidovudine content (British Pharmacopoeia Commission, 1996; Council of Europe, 1997; US Pharmacopeial Convention, 1998b).

A simple, rapid method by high-performance liquid chromatography (HPLC) for zidovudine in plasma with a reversed-phase column and detection at 265 nm has been reported. The detection limits in plasma and urine were 20 and 200 ng/mL, respectively. Quantification of zidovudine in serum, milk and tissues by isocratic HPLC has been reported. A colorimetric method of assay has been developed for anti-human immunodeficiency virus (HIV) agents, including zidovudine. Simultaneous quantification of zidovudine and its metabolites in serum and urine by HPLC with a column-switching technique has been reported. The concentration of zidovudine in serum has been measured by the enzyme-linked immunosorbent assay and by the time-resolved fluoroimmunoassay. Paper and thin-layer chromatography of nucleoside derivatives including zidovudine have also been used (Sethi, 1991).

1.2 Production

Zidovudine was first synthesized in 1964. It was prepared by mesylation of 1′-(2′-deoxy-5′-*O*-trityl-β-D-lyxosyl)thymine to the sulfonate, which was treated with lithium azide in *N*,*N*-dimethylformamide to form 3′-azido-3′-deoxythymidine (Sethi, 1991). Other synthesis methods have been reported (Glinski *et al.*, 1973; Chu *et al.*, 1988).

Information available in 1999 indicated that zidovudine was manufactured and/or formulated in 35 countries (CIS Information Services, 1998; Royal Pharmaceutical Society of Great Britain; 1999; Swiss Pharmaceutical Society, 1999).

1.3 Use

Zidovudine was originally synthesized in the 1960s as a possible anti-cancer agent, but was found to be ineffective. In 1985, it was found to be active against HIV-1 *in vitro* (Hoetelmans *et al.*, 1996). It was approved as the first anti-HIV agent in 1987 and remains a widely prescribed mainstay of HIV therapy. Zidovudine has additive or synergistic activity with almost all other antiretroviral agents except the chemically related stavudine (3′,5′-didehydrodideoxythymidine) with which it is antagonistic. Zidovudine alone provides only transient benefit (Graham *et al.*, 1992; Concorde Coordinating Committee, 1994; Vella *et al.*, 1994) owing to the development of genotypic changes within the virus that result in relative resistance to zidovudine (Larder &

Kemp, 1989; Larder *et al.*, 1989). Prolonged administration of zidovudine as part of a regimen which does not suppress HIV replication can lead to selection of strains of HIV that contain mutations which confer broad cross-resistance to all nucleoside reverse transcriptase inhibitors (Shafer *et al.*, 1995; Venturi *et al.*, 1999).

Zidovudine is frequently included in a variety of highly active antiretroviral regimens such as with lamivudine and indinavir (a protease inhibitor), with lamivudine and nevirapine or efavirenz, and with lamivudine and abacavir (Hammer *et al.*, 1997; Staszewski *et al.*, 1998; Barry *et al.*, 1999). Zidovudine plus didanosine is one of the most potent, extensively studied nucleoside regimens (Husson *et al.*, 1994; Henry *et al.*, 1998; Montaner *et al.*, 1998), although it is not superior to didanosine plus 3′,5′-didehydroxydideoxythimidine (Raffi *et al.*, 1998).

Perhaps the most dramatic use of zidovudine was that of the Pediatric AIDS Clinical Trials Group in its protocol 076, which showed that giving zidovudine alone during the last two trimesters of pregnancy, intravenously intrapartum and to the newborn orally could decrease the incidence of vertical transmission of HIV by two-thirds (Connor *et al.*, 1994; Simonds *et al.*, 1998). Subsequent studies have demonstrated that shorter durations and simpler all-oral regimens still provide substantial benefits (Wade *et al.*, 1998; Dabis *et al.*, 1999; Shaffer *et al.*, 1999; Wiktor *et al.*, 1999). Several studies have demonstrated that administration to pregnant women of zidovudine-containing combinations that reduce maternal HIV viral loads to near zero is highly likely to prevent vertical transmission (Garcia *et al.*, 1999; Mofenson *et al.*, 1999). The US Public Health Service has published guidelines for the treatment of HIV in pregnancy (Centers for Disease Control and Prevention, 1998a).

Zidovudine is also used in combination with other agents for the prevention of HIV after exposure to the virus (Tokars *et al.*, 1993; Katz & Gerberding, 1997; Centers for Disease Control and Prevention, 1998b).

It appears to be effective in the prevention and possibly the treatment of AIDS dementia complex and paediatric HIV developmental disability (Pizzo *et al.*, 1988; Portegies *et al.*, 1989; Bacellar *et al.*, 1994; Baldeweg *et al.*, 1995). It is also effective in HIV-related thrombocytopenia (Hirschel *et al.*, 1988).

1.4 Occurrence

Zidovudine is not known to occur as a natural product. No data on occupational exposure were available to the Working Group.

1.5 Regulations and guidelines

Zidovudine is listed in the British, European, French, German, Swiss and US pharmacopoeias (Swiss Pharmaceutical Society, 1999).

2. Studies of Cancer in Humans

2.1 Ecological studies

Coté and Biggar (1995) linked data from AIDS and cancer registries in several parts of the USA to compare the risk for non-Hodgkin lymphoma among people with AIDS before and after introduction of zidovudine. Each patient was followed-up for a maximum of 3.5 years. The standardized incidence ratios for non-Hodgkin lymphoma among patients with AIDS relative to that of the background population without AIDS were 222 (95% confidence interval [CI], 190–260) in 1981–86 and slightly lower (193; 95% CI, 176–212) in 1988–90 when zidovudine and other antiretroviral agents were on the market. [The Working Group noted that information on the treatment received by the patients was not available, but it has been estimated that 30–50% of those treated in the latter era would have received zidovudine (Gail *et al.*, 1990).]

2.2 Cohort studies

Pluda *et al*. (1990) examined the records of 55 patients with AIDS or severe AIDS-related complex (defined as having either oral candidiasis, oral hairy leukoplasia (Pluda *et al.*, 1993) or weight loss of > 10% of total body weight) who during 1985–87 were entered into phase I studies, in the Clinical Oncology Program of the National Cancer Institute, USA, of zidovudine alone (n = 29), zidovudine with simultaneously administered aciclovir (n = 8) or zidovudine alternated with zalcitabine (n = 18). All patients had fewer than 350 CD4 cells/μL plasma at the time of entry. Patients were followed for a minimum of 0.5 years and a maximum of 3.1 years, during which time eight patients developed a high-grade non-Hodgkin lymphoma of the B-cell type (three out of 29 in the group receiving zidovudine alone, three out of eight receiving zidovudine plus aciclovir and two out of 18 receiving zidovudine alternated with zalcitabine). When the follow-up was extended by another 1.7 years to a maximum of 4.8 years (Pluda *et al*., 1993), no additional cases of lymphoma were seen. The estimated cumulative risk of developing non-Hodgkin lymphoma by 24 months of therapy was 12% (95% CI, 4.7–27%; Kaplan-Meier method), increasing to 29% (95% CI, 15–49%) by 36 months. The median CD4 cell count at initiation of antiretroviral therapy in the lymphoma patients was 26 cells/μL (range, 8–135) (Pluda *et al.*, 1990).

In a randomized, placebo-controlled trial, Fischl *et al*. (1990) included 711 subjects with mildly symptomatic HIV infection diagnosed at one of 29 treatment centres in the USA during the period 1987–89. Of these, 351 subjects were assigned to receive placebo and 360 were assigned to receive 200 mg zidovudine orally every 4 h (six did not receive zidovudine). All subjects had to have a mean pretreatment CD4 count of > 200 but < 800 cells/μL plasma. The median duration of follow-up was 11 months (range, 0.3–23 months), during which time one case of non-Hodgkin

lymphoma of the B-cell type was seen in each group, and three cases of Kaposi sarcoma were seen in the group given placebo and two in the group given zidovudine.

Moore *et al.* (1991a) analysed data from a prospective, observational multicentre study at 12 clinical sites in the USA of 1030 patients with AIDS or advanced AIDS-related complex (at least one of five symptoms of HIV infection: oral thrush, weight loss of ≥ 10%, unexplained fever, diarrhoea or herpes zoster). All the patients had a CD4 cell count of no more than 250 cells/μL plasma at the time of entry. All patients were treated with zidovudine only at a daily dose of 1200 mg (85% of patients) or 600 mg (15%). Data collection was completed at baseline, i.e. during the period 1987–88, and every two months for two years or until death (median time, 1.5 years). Twelve patients had non-Hodgkin lymphoma, and two additional patients were suspected of having developed the disease before the date of start of zidovudine treatment and were excluded from the analysis. Twenty-four patients developed non-Hodgkin lymphoma after the start of treatment; the latter observation was equivalent to a rate of 1.6 cases of non-Hodgkin lymphoma per 100 person–years of therapy. The cumulative risk for non-Hodgkin lymphoma over the two years of zidovudine therapy showed a linear increase of 0.8% for each additional six months of therapy. Neither the proportion of time during the study that zidovudine was received by the patients nor the average daily dose was associated with non-Hodgkin lymphoma. [The Working Group noted that the power of the study to detect differences between the two dose groups was very low.]

In an international, randomized trial in 175 centres in several countries between 1992 and 1994, the Delta Coordinating Committee (1996) allocated 3207 individuals with antibodies to HIV who had symptoms of infection or a CD4 count of < 350 cells/μL plasma to treatment with either zidovudine at 600 mg per day (n = 1055), zidovudine (same dose) plus didanosine at 400 mg per day (n = 1080) or zidovudine (same dose) plus zalcitabine at 2.25 mg per day (n = 1072). The patients were followed up to September 1995 for a median of 2.5 years (range, 1.8–2.9), during which time 14 deaths due to cancer [not further specified] occurred; five of the deaths occurred in the group treated with zidovudine alone, five in the group treated with zidovudine plus didanosine and four in the group treated with zidovudine plus zalcitabine. [The Working Group noted that these trials were designed to evaluate the efficacy of zidovudine in the treatment of patients with various degrees of severity of immunosuppression. For the purposes of evaluating cancer risk, therefore, the numbers of participants were too small and the length of follow-up too short, cancer incidence may have been under-ascertained, and cancer rates could not be analysed adequately.]

In a study of 2627 HIV-infected homosexual men who were followed prospectively in four sites in the USA during 1985–91, Muñoz *et al.* (1993) examined the effects of severity of immunosuppression, assessed by CD4 cell counts, and antiretroviral therapy, mainly zidovudine, on the incidence of Kaposi sarcoma and non-Hodgkin lymphoma, whenever these malignancies constituted the AIDS-defining disease. On the basis of a total of 194 cases of Kaposi sarcoma and 34 non-Hodgkin

lymphomas observed among the AIDS-free seropositive individuals, the authors found a strong inverse relationship between the CD4 cell count and the risk for Kaposi sarcoma and a moderate inverse relationship between this cell count and the risk for non-Hodgkin lymphoma. In a further multivariate analysis with adjustment for severity of immunosuppression and other factors, the authors observed non-significantly decreased relative risks for Kaposi sarcoma (relative risk, 0.83) and non-Hodgkin lymphoma (relative risk, 0.47) associated with prophylactic use of antiretroviral agents. [The Working Group noted that the type of antiretroviral therapy used is not specified, although it would have been predominantly zidovudine.]

Forseter *et al*. (1994) conducted a prospective, observational study of 60 health-care workers treated at one institution in New York City, USA, between 1989 and 1992, who had percutaneous or permucosal exposures to blood or body fluids of HIV-infected patients. Although study subjects were recommended a 42-day course of therapy with 200 mg zidovudine every 4 h, starting from 30 min to three days after exposure, only 21 completed the course. Among the 42 subjects followed for three months or longer (mean, 7.8 months; range, three months to 2.7 years), none had undergone HIV antibody seroconversion. One 23-year-old health-care worker reported the development of Hodgkin disease (nodular sclerosing variety, stage 3A) 1.5 years after completion of the 42-day course of zidovudine therapy. [The Working Group noted the anecdotal nature of the report.]

2.3 Case–control studies

Levine *et al.* (1995) reported the results of a population-based case–control study of 112 cases of intermediate or high-grade non-Hodgkin lymphoma diagnosed in 1989–92 among HIV-infected homosexual or bisexual men living in Los Angeles County, USA. The cases were identified in 1431 patients notified with an intermediate- or high-grade lymphoma during the period to the population-based cancer registry of Los Angeles. Of these, 658 died before an interview could be conducted. Of the remaining cases, 527 participated in the study of non-Hodgkin lymphoma, and each gave a blood sample for determination of HIV status. Personal interviews were conducted with a structured questionnaire to obtain information on the subjects' lifetime history of use of medications ever taken for at least a month, including zidovudine and other antiretroviral agents, medical history and selected personal habits and lifestyle factors. For each of the 112 HIV-positive homosexual or bisexual men who provided information about zidovudine, one matched HIV-positive asymptomatic control subject was selected from a County- or University-affiliated clinic in the community. The matching criteria were year of birth (within three years), race and ethnic group, sex, language of interview and mode of transmission of initial HIV infection. Data on zidovudine use and the other factors investigated were obtained from controls in a similar way to cases. The reference period for cases was up to 12 months before the time of diagnosis, and for controls up to 12 months before the time

of diagnosis of the matched case. Forty-four men with lymphoma reported a history of zidovudine use for at least a month (mean duration, 19 months; SD, 13 months) compared with 24 controls (21%: mean duration, 12.6 months; SD, 10.5 months). The matched odds ratio for lymphoma associated with use of zidovudine was 1.6 (95% CI, 0.94–2.9). The authors noted that until around 1990 zidovudine use was primarily restricted to patients with AIDS or symptomatic HIV disease, whereas after that time it became accepted practice to use this drug in asymptomatic HIV-positive subjects with fewer than 500 CD4 cells/μL. Therefore, it is possible that the comparison of cases with asymptomatic controls may have provided a misleading estimate of association. For this reason, zidovudine use by 49 men with AIDS-associated lymphoma was compared with use of the drug by individually matched controls in whom AIDS was diagnosed in the same period but who did not have a lymphoma. These controls were identified from the records of one treatment centre; the same matching criteria were used as described for the comparison with asymptomatic controls. Twelve (about 25%) of these men had used zidovudine (mean duration, 13 months; SD, 10.5 months) compared with 20 controls (41%; mean duration, 11 months; SD, 7.1 months). The matched odds ratio was 0.43 (95% CI, 0.17–1.1). [The Working Group noted that the proportion of patients with lymphoma who were HIV-positive and died before interview was unknown, and this may have have biased the association with reported zidovudine use. The participation rate among control subjects was not specified. Although CD4 counts were not available, restriction of the analysis to AIDS cases was an attempt to address this limitation.]

2.4 Childhood cancer

The medical records of prospectively followed HIV-exposed infants with known exposure to zidovudine *in utero* and/or neonatallly were reviewed by Hanson *et al.* (1999) for the development of tumours. The HIV-infected pregnant mothers were enrolled from various treatment centres across the USA during 1989–96, and 727 live-born children, of whom 115 were also included in the study of Sperling *et al.* (1998), described in section 4.3, were followed from date of birth onwards, for a mean of 1.2 or 3.2 years (range, one month to six years). No tumours of any type were reported in the 727 zidovudine-exposed infants; however, on the basis of the incidence rates for cancer in the general childhood population in the USA the authors calculated an upper 95% CI of 18. [The Working Group noted that a substantially increased risk could not be excluded.]

In two studies described in detail in section 4.3, no tumours were observed in the children of mothers given zidovudine during pregnancy. The length of follow-up was 1.5 years in one study (Sperling *et al.*, 1998) and 4.2 years in the other (Culnane *et al.*, 1999).

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

Groups of 100 male and 100 female CD-1 mice, seven weeks of age, were given zidovudine free base [purity not specified] in 0.5% methylcellulose once daily by gavage at a dose of 30, 60 or 120 mg/kg bw per day. Twenty-five to 40 mice from each group were used only for haematological examinations and for determinations of the plasma concentration of the drug. At day 91, anaemia was seen in animals at the intermediate and high doses, and the doses were lowered to 20, 30 and 40 mg/kg bw per day. Two separate groups of 85 male and 85 female mice were left untreated or were given the vehicle alone. The study was terminated at 19 and 22 months for male and female mice, respectively. Tissues from all mice in the untreated, vehicle control and high-dose groups were examined microscopically. In addition, the vaginas from all mice at the low and intermediate doses were examined. Treatment with zidovudine did not affect the survival rate in either sex, and the rate at 18 months was 50%. Body weight was unaffected by treatment in either sex. The incidences of vaginal squamous-cell carcinomas were 0/60, 0/60, 0/60, 0/60 and 5/60 [$p = 0.06$, Fisher's exact test] in untreated controls, vehicle controls and at the three doses, respectively. One squamous-cell papilloma of the vagina was seen at the intermediate dose and one at the high dose. Squamous-cell hyperplasia of the vaginal epithelium was seen in all groups of mice, including the controls, and the incidence of moderate to severe hyperplasia was dose-relatedly increased in mice given the intermediate or high dose of zidovudine. Treatment did not affect the incidence of any other benign or malignant tumour in any tissue or organ examined [specific tumour incidences not reported] (Ayers *et al*., 1996a).

Groups of 95 male and 95 female $B6C3F_1$ mice, seven to eight weeks of age, were treated with zidovudine (~98% pure) in 0.5% methylcellulose by gavage at a daily dose of 0, 30, 60 or 120 mg/kg bw, administered as two equal doses at least 6 h apart, on five days per week for 105 weeks. Each group of 95 animals of each sex comprised 50 animals of each sex for evaluation of carcinogenic response, 30 animals of each sex for evaluation of haematological end-points and bone-marrow cellularity, and 15 animals of each sex from which blood was drawn for determination of the plasma concentrations of zidovudine at week 54. The survival and mean body weights of mice exposed to zidovudine were similar to those of the vehicle control groups. Squamous-cell carcinomas or papillomas (combined) of the vagina occurred in 0/50, 0/49, 5/45 ($p = 0.028$) and 11/49 ($p < 0.001$) animals in the control group and at the three doses, respectively ($p < 0.001$, trend test), and epithelial hyperplasia of the vagina was observed in 0/50, 3/49, 4/45 and 11/49 ($p < 0.01$) mice in those groups, respectively. Three renal tubular adenomas and one renal tubular carcinoma were observed in male mice receiving the high dose, and the combined incidence in this group exceeded the

range in historical controls: 2/365 (range, 0–4%). The incidences of Harderian gland tumours in male mice were 3/50, 5/50, 2/50 and 10/50 ($p = 0.059$) in the four groups, respectively ($p = 0.027$, trend test) (National Toxicology Program, 1999). [The Working Group noted that the incidence of squamous-cell vaginal tumours in unexposed mice is very rare.]

3.1.2 *Rat*

Groups of 60 male and 60 female CD rats, six weeks of age, were given zidovudine [purity not specified] in 0.5% methylcellulose once daily by gavage at a dose of 80, 220 or 600 mg/kg bw. At day 91, the high dose was lowered to 450 mg/kg bw per day because of the occurrence of anaemia. Progression of anaemia led to a further reduction of the high dose to 300 mg/kg bw per day on day 278. Two separate groups of 60 male and 60 female rats were left untreated or were given the vehicle alone. The study was terminated at 24 and 22 months for male and female rats, respectively. Tissues from all rats in the untreated, vehicle control and high-dose groups were examined microscopically. In addition, the vaginas from all female rats at the low and intermediate doses were examined. Treatment with zidovudine did not affect the survival rate in either of the sexes, and the rate at 18 months was 50% or greater. Body weight was unaffected by treatment in either sex. Two squamous-cell carcinomas of the distal vagina were observed in females at the high dose, but no vaginal tumours occurred in the other groups, or in the untreated or vehicle control groups. Treatment with zidovudine did not affect the incidence of any other benign or malignant tumour in any tissue or organ examined [specific tumour incidences not reported] (Ayers *et al*., 1996a). [The Working Group noted that the occurrence of squamous-cell vaginal tumours in unexposed rats is exceedingly rare.]

3.2 Transplacental exposure

Mouse: Groups of 60 female CD-1 mice, 79 days of age, were mated with male CD-1 mice and given zidovudine [purity not specified] in 0.5% methylcellulose once daily by gavage at 20 or 40 mg/kg bw per day, beginning on day 10 of gestation and throughout gestation, parturition and lactation. At weaning, zidovudine was administered to the offspring at the same doses in the drinking-water for 17–35 days and then by gavage for 24 months. Two additional groups were treated similarly with 40 mg/kg bw per day, but one group was treated only until day 21 of lactation and the second by gavage for 90 days after birth. Two groups each of 60 female mice were either untreated or were given the vehicle, beginning on day 10 of gestation and throughout gestation, parturition and lactation, and then in the drinking-water for 17–35 days, followed by daily gavage for 24 months. The study was designed to give a total of 70 male and 70 female progeny in each dose group. All animals were killed at 24 months. Fifty separate tissues and organs [not specified] were taken for micro-

scopic examination, and the vaginas from all females were examined microscopically. There was no treatment-related effect on survival rates or body weight. No treatment-related increase in the incidence of neoplastic or non-neoplastic lesions was observed in males [specific tumour incidences not reported]. In females, vaginal squamous-cell carcinomas were found in 11 ($p = 0.0002$) mice at the high dose, in two mice at the low dose and in one mouse given the high dose by gavage for 90 days after birth. No vaginal tumours were seen in the other groups (Ayers *et al*., 1997).

Groups of female CD-1 Swiss mice [age unspecified] were mated with male CD-1 mice, and 45 pregnant mice were given zidovudine (purity, > 99.8%) by gavage at doses of 0 (17 litters), 12.5 (13 litters) or 25 (15 litters) mg/mouse (approximately 210 and 420 mg/kg bw for the low and high dose, respectively) on days 12–18 of gestation. The pups were delivered normally and were kept without further treatment. Ten pups of each sex from each group were killed 13, 26 and 52 weeks after delivery. At week 52, the observation of lung and liver tumours prompted the authors to kill additional mice and to report the results. The numbers of mice in each group were 31 male controls and 23 and 26 at the low and high doses and 30 female controls and 22 and 24 at the low and high doses. In the two sexes combined, the incidence of lung carcinomas was 3% in controls, 7% at the low dose and 14% at the high dose ($p = 0.037$, Cochran-Armitage trend test). The multiplicity of lung tumours was 0.10 in male controls and 0.13 and 0.50 at the low and high doses ($p = 0.014$, trend test) and 0.13 in female controls and 0.14 and 0.38 at the low and high doses ($p = 0.15$, trend test). Neoplasms of the ovary, uterus and vagina were seen in 0% of controls, 14% at the low dose and 17% at the high dose ($p = 0.033$, trend test). The incidence of hepatocellular tumours (mainly adenomas) was increased in males, being about 13% in controls, 30% at the low dose and 52% at the high dose; the multiplicity of hepatocellular tumours was 0.23 in controls, 0.48 at the low dose and 0.79 at the high dose ($p = 0.013$, trend test) (Olivero *et al*., 1997).

In a study involving prenatal initiation and postnatal promotion, groups of pregnant CD-1 Swiss mice were given 0 or 25 mg/mouse (approximately 420 mg/kg bw) zidovudine (purity, > 99.8%) by gavage once daily on days 12–18 of gestation. Groups of 16 male and 14 female control pups and 13 male and 17 female pups of zidovudine-treated dams were selected randomly at five weeks of age for topical treatment with 12-*O*-tetradecanoylphorbol 13-acetate (TPA) on the dorsal skin at a dose of 2 µg twice a week for 17 weeks and then 5 µg twice a week in weeks 18–35. The experiment was terminated 41 weeks after the start of TPA promotion, at which time the offspring were 46 weeks of age. At the end of the experiment, about 96% of the mice given zidovudine plus TPA had developed skin tumours. The effect was more pronounced in females: 5/14 (35%) female mice given TPA and 15/17 (88%) given zidovudine plus TPA had skin tumours ($p < 0.05$). The average numbers of tumours per mouse was 0.57 ± 0.13 (mean ± SE) in the group given TPA alone and 1.44 ± 0.36 in that given zidovudine plus TPA ($p = 0.006$). Most (82%) of the skin tumours were papillomas; the rest (18%) were keratoacanthomas (Zhang *et al*., 1998).

3.3 Intravaginal administration

Mouse: Groups of 50 female CD-1 mice, seven weeks of age, were given zidovudine [purity not specified] in 0.9% saline intravaginally twice daily approximately 6 h apart at a dose of 2 or 8 mg/day. Two additional groups of 50 female mice were either left untreated or were given the vehicle intravaginally. The study was terminated at 24 months. The vagina and cervix from animals in all groups were examined microscopically. Vaginal squamous-cell carcinomas were observed in 2/50 mice at the low dose and 13/50 at the high dose [$p < 0.001$]. Vaginal epithelial-cell tumours were not seen in either control group (Ayers *et al*., 1996a).

3.4 Administration with other agents

Mouse: Groups of 50 male and 50 female $B6C3F_1$ mice, seven to eight weeks of age, were treated with zidovudine (~98% pure) in 0.5% aqueous methyl cellulose by gavage at a daily dose of 0 (control), 30, 60 or 120 mg/kg bw in two equal doses on five days per week for 105 weeks. All groups also received subcutaneous injections of 500 or 5000 U α-interferon three times per week for 105 weeks. Survival rates and body weights were similar in treated and vehicle control groups. The incidences of squamous-cell carcinoma of the vagina in the groups receiving 500 U α-interferon were 0/49, 0/44, 5/48 ($p = 0.030$) and 6/48 ($p = 0.011$, logistic regression test), in the control group and at the three doses, respectively ($p < 0.001$, trend test). Epithelial hyperplasia of the vagina was seen in 0/49 controls and 4/44, 8/48 and 12/48 at the three doses, respectively ($p = 0.047$, trend test). In the groups receiving 5000 U α-interferon, the incidences of squamous-cell carcinoma or papilloma (combined) of the vagina were 1/50, 1/48, 5/48 and 4/50 ($p = 0.047$, trend test), and those of epithelial hyperplasia of the vagina were 1/50, 4/48, 8/48 and 15/50 ($p < 0.01$, trend test) in controls and at the three doses, respectively. There was no significant increase in the incidence of tumours at other sites (National Toxicology Program, 1999).

4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Absorption, distribution, metabolism and excretion

4.1.1 *Humans*

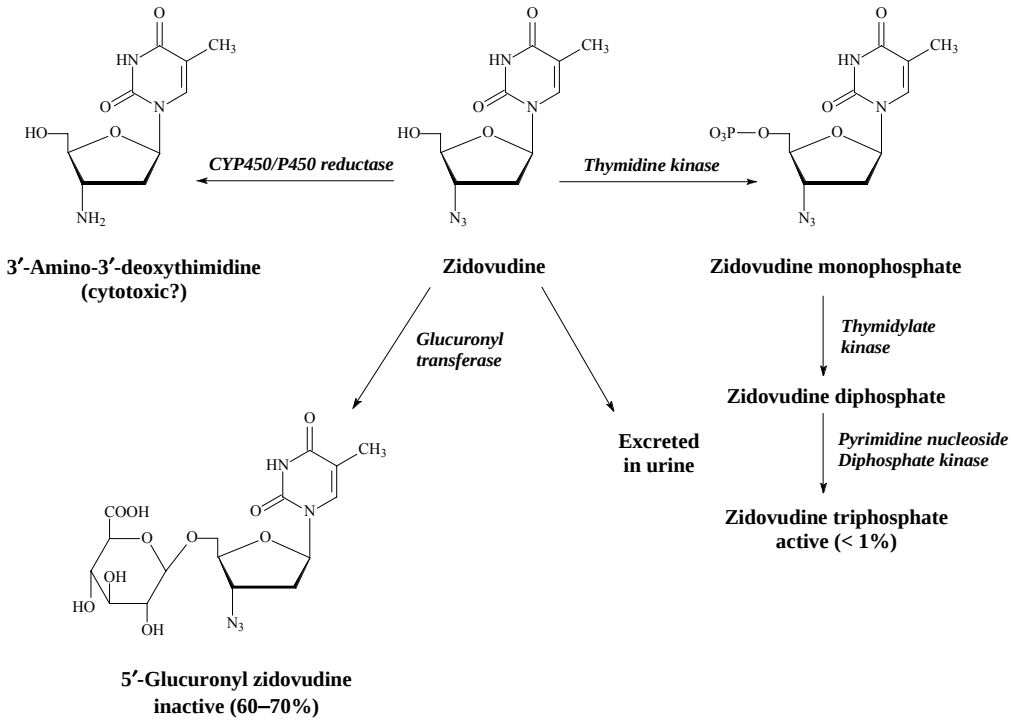
The pharmacokinetics of zidovudine has been reviewed and summarized extensively (Morse *et al*., 1993; Dudley, 1995; Acosta *et al*., 1996; Hoetelmans *et al*., 1996), and large inter- and intra-individual variations have been observed (Mentré *et al*, 1993; Dudley, 1995; Acosta *et al*., 1996; Hoetelmans *et al*., 1996). The peak

plasma concentrations of zidovudine after a dose of 200 mg have been reported to be 3.2–10.8 μmol/L [0.86–2.9 mg/L], and are reached after 30–60 min (Dudley, 1995; Hoetelmans *et al.*, 1996). The half-time for removal of the drug from plasma is about 1 h, and the clearance rate is 5–12.5 L/h (Morse *et al*., 1993; Dudley, 1995). In one study of HIV-negative individuals given an intravenous dose of 2.5 mg/kg bw zidovudine, the integrated area under the curve of plasma concentration–time was 22% of the total for zidovudine, 72% for 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine and 5% for 3′-amino-3′-deoxythymidine, and the half-time for removal was 1.2 h for zidovudine, 1.7 h for 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine and 2.7 h for 3′-amino-3′-deoxythymidine (Stagg *et al*., 1992). The renal clearance rate has been reported to be about 12 L/h for zidovudine and 18 L/h for 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine (Morse *et al*., 1993). These values are reduced in patients with compromised renal function (Dudley, 1995; Acosta *et al*., 1996). In patients with normal kidney and liver function, the pharmacokinetics of zidovudine is similar after the first dose and during long-term dosing (Gallicano *et al.*, 1993), but significant changes may occur in the presence of hepatic or renal compromise (Dudley, 1995; Acosta *et al*., 1996; Hoetelmans *et al*., 1996). The pharmacokinetics of zidovudine in cerebrospinal fluid has been reported (Rolinski *et al*., 1997); the drug penetrated the cerebrospinal fluid slowly, the peak concentration being achieved at 2 h, an area under the curve that was about 75% of that in plasma and a half-time of about 3 h.

The absorption, distribution, metabolism and excretion of zidovudine in adults with and without HIV infection have been reviewed extensively (Kamali, 1993; Morse *et al*., 1993; Dudley, 1995; Stretcher, 1995; Acosta *et al*., 1996; Hoetelmans *et al*., 1996). Oral dosing was used in the majority of these studies, and absorption was significantly altered by the presence of food in the stomach (Acosta *et al*., 1996). About 64% of an oral dose is bioavailable, although zidovudine binds poorly to plasma proteins (~25%) and is distributed to cells by passive diffusion (Kamali, 1993; Dudley, 1995; Acosta *et al*., 1996). The drug is distributed throughout the body and has been found in plasma, saliva, semen, breast milk and cerebrospinal fluid, although the concentration in the last may be only 15% of that in plasma (Morse *et al*., 1993; Dudley, 1995; Acosta *et al*., 1996).

Zidovudine is metabolized primarily along three separate pathways (Figure 1), and about 95% of a total dose is recovered in the urine, with 15–20% as unchanged drug (Stagg *et al*., 1992; Morse *et al*., 1993; Dudley, 1995). The major pathway is first-pass glucuronidation with renal excretion and results in the elimination of about 65–75% of the total dose. The urinary glucuronide metabolite, 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine, is formed by the action of uridine 5′-diphosphoglucuronyl transferase and was first characterized chemically by Good *et al*. (1990). In plasma and urine, the 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine:zidovudine ratio is typically 3 or 4, although large interindividual variation has been reported (Acosta *et al*., 1996).

Figure 1. Metabolic pathways of zidovudine



From Veal & Back (1995)

A second pathway involves the action of various hepatic CYP450 oxidases and reductases, resulting in the production of a toxic metabolite, 3′-amino-3′-deoxythymidine (Stagg *et al*., 1992; Acosta *et al*., 1996). This metabolite is formed to varying extents in different tissues and represents about 2% of the total dose in the urine. It has a longer plasma half-life than either zidovudine or 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine (Stagg *et al*., 1992). In one study (Hoetelmans *et al*., 1997) of samples from 23 patients, the amount of 3′-amino-3′-deoxythymidine in cerebrospinal fluid was about 1.8% that of zidovudine, and similar ratios were found in plasma.

The metabolic pathway responsible for the antiviral activity is phosphorylation (Figure 1). The mono-, di- and triphosphates of zidovudine are formed rapidly through the action of thymidine kinase, thymidylate kinase and pyrimidine nucleoside diphosphate kinase, respectively (Stretcher, 1995; Veal & Back, 1995; Acosta *et al*., 1996; Hoetelmans *et al*., 1996; Peter & Gambertoglio, 1998). Since zidovudine is a good substrate for thymidine kinase, with 60% of the maximal velocity (V_{max}) of thymidine, zidovudine monophosphate accumulates and typically comprises about 90% of the total intracellular zidovudine phosphates in healthy individuals (Hoetelmans *et al*., 1996; Peter & Gambertoglio, 1998). Zidovudine diphosphate and zidovudine triphosphate are

present in equal proportions of ~5% (Peter & Gambertoglio, 1998). There is evidence (Stretcher, 1995; Acosta *et al*., 1996; Peter & Gambertoglio, 1996) that this balance is shifted in HIV-positive patients with CD4 counts between 300 and 500/mm^3, in whom zidovudine monophosphate constitutes about 74% of the total phosphates and zidovudine diphosphate and zidovudine triphosphate each account for about 13%. The difference may be due to the viral infection, since short-term and long-term exposure to zidovudine produced similar results (Peter & Gambertoglio, 1996). In patients, the zidovudine monophosphorylation pathway saturates after each dose of 100 mg or more, suggesting that monophosphorylation is largely independent of current clinical doses. Conversion to di- and triphosphates is more closely related to individual phosphorylation capacity (Stretcher, 1995). Zidovudine triphosphate is eventually incorporated into DNA, resulting in the termination of replication (Morse *et al*, 1993; Veal & Back, 1995; Peter & Gambertoglio, 1998). The antiviral activity of the drug is thought to result from direct inhibition of viral reverse transcriptase and truncation of proviral DNA replication (Peter & Gambertoglio, 1998). Although critical to the mechanism of antiviral activity, zidovudine phosphorylation is responsible for only a small fraction (~1%) of the total disposition of the drug.

After a woman received zidovudine intravenously by continuous infusion (0.12 mg/kg bw per h) 2 h before delivery, her blood contained 0.28 μg/mL and the blood of her newborn contained 0.27–0.51 μg/mL 6–48 h after birth. 3′-Azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine was present at a concentration of 1.12 μg/mL in the mother and 1.1–2.31 μg/mL in the infant (Chavanet *et al*., 1989).

O'Sullivan *et al*. (1993) studied the pharmacokinetics of zidovudine in eight near-term pregnant women given zidovudine orally at about 154 mg/kg bw per day before delivery. The mean body clearance rate was 26 ± 10 mL/min per kg bw and the half-time for elimination was 1.3 ± 0.2 h. The half-time for elimination in amniotic fluid was 13 ± 0.58 h, which was 10 times longer than in the mother.

4.1.2 *Experimental systems*

The absorption, distribution, metabolism and excretion of zidovudine have been determined in mice (Trang *et al*., 1993; Manouilov *et al*., 1995; Chow *et al*., 1997, 1998), rats (de Miranda *et al*., 1990) and rhesus monkeys (Boudinot *et al*., 1990; Cretton *et al*., 1991). The basic metabolic pathways of glucuronidation, phosphorylation and reduction to 3′-amino-3′-deoxythymidine are similar in all these species, but the bioavailability and pharmacokinetics vary. Absorption, distribution and elimination are more rapid in rodents than in humans, and the bioavailability is greater in rats and mice than in primates. Non-human primates appear to be excellent human surrogates because the pharmacokinetics of zidovudine, including clearance and steady-state volume of distribution, is virtually identical to that in humans.

In female B6C3F$_1$ mice given doses of 15, 30 and 60 mg/kg bw zidovudine by gavage, the maximum serum concentrations of 9, 18 and 40 μg/mL, respectively, were

reached at 15–22 min (Trang *et al*., 1993). The half-time for elimination was 16–22 min. The absolute bioavailability was estimated to be 86%. The disposition of zidovudine followed a one-compartment open model, with first-order absorption and elimination after oral dosing and first-order elimination after intravenous injection. The pharmacokinetics of zidovudine in mice was not dependent on the route of administration.

Concentrations of zidovudine were determined in plasma and lymph nodes of female NIH Swiss mice given 50 mg/kg bw zidovudine intravenously, intraperitoneally or orally (Manouilov *et al*., 1995). The maximum serum concentrations of zidovudine were obtained after about 5 min with all routes, and the half-time for removal was 23–46 min. The absolute bioavailability was 49% after oral administration and 100% after administration by the other two routes. The concentrations of zidovudine in the neck, axillary and mesenteric lymph nodes reached about 30% of those in serum.

Female C57BL/6 mice, with and without retroviral infection, were given a single subcutaneous dose of 25 mg/kg bw [^{3}H]zidovudine, and tissues were examined after 30, 60 and 90 min for concentrations of zidovudine and zidovudine triphosphate (Chow *et al*., 1997). Healthy and infected animals showed a similar pattern of distribution in 10 organs, the highest concentrations being found in kidney and muscle and the lowest in thymus, lymph nodes and brain. In uninfected mice, the highest concentrations of zidovudine monophosphate were observed in the bone marrow, kidney and spleen and the lowest in thymus, lymph nodes and brain, although infected mice had relatively high concentrations of zidovudine triphosphate in lymph nodes. In similarly designed studies (Chow *et al*., 1998), mice were given subcutaneous injections of 25 mg/kg bw zidovudine twice daily for eight weeks. The profiles of zidovudine and zidovudine monophosphate were generally similar to those observed in the single-dose experiments; however, after multiple injections, zidovudine triphosphate accumulated preferentially in the spleen and bone marrow owing to the high cell density in those organs.

The tissue disposition and metabolism of zidovudine were studied in rats given a single dose of 10 mg/kg bw [^{3}H]zidovudine by gavage (de Miranda *et al*., 1990). The drug was absorbed and distributed rapidly in all tissues, with peak concentrations occurring by 15 min. The concentrations of zidovudine and its metabolites (zidovudine equivalents) in most tissues were similar to or higher than those in plasma, but very low concentrations were observed in brain, suggesting slow cerebrospinal fluid uptake. The disappearance of radiolabel from blood and plasma was biphasic. Urinary (78%) and faecal (21%) excretion of the drug was complete by 24 h. In urine, 88% of radiolabel was associated with unchanged drug and the remainder with five metabolites. 3′-Azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine and 3′-amino-3′-deoxythymidine were detected in both plasma and urine, and 7% of the radioactive dose was excreted, primarily as 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine, in the bile. The major faecal metabolite (> 70%) was 3′-amino-3′-deoxythymidine.

Rhesus monkeys received oral doses of 60 or 200 mg/kg bw zidovudine and an intravenous dose of 60 mg/kg bw. The bioavailability was 92% after the lower oral

dose and about 50% after the higher dose, and the plasma half-time was 30–45 min at the lower dose and 80–120 min at the higher dose. Diffusion of zidovudine into cerebrospinal fluid was much slower than that into plasma, and the peak concentration in cerebrospinal fluid was 5–21% of that in plasma (Boudinet *et al.*, 1990).

After administration of 33.3 mg/kg bw zidovudine to rhesus monkeys by subcutaneous injection, the maximum concentrations of zidovudine, 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine and 3′-amino-3′-deoxythymidine in plasma were found at 45 min, with half-times of 1 h for zidovudine and the glucuronide and 1.6 h for 3′-amino-3′-deoxythymidine. In the urine, about 27% of the total dose was excreted as zidovudine, 60% as 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine and 1.8% as either 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine or 3′-amino-3′-deoxythimidine within the first 24 h. The average peak concentrations of zidovudine in the cerebrospinal fluid were 30% of those observed in plasma, but were highly variable between monkeys, and very little 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine was recovered from the cerebrospinal fluid (Cretton *et al.*, 1991).

Perfused human placentas were used to show that zidovudine readily crosses the human placenta and that the passage is bidirectional, with no evidence of active or carrier-mediated transport. No evidence for glucuronide conjugation of zidovudine by the placenta was reported (Schenker *et al*., 1990).

Zidovudine added at a concentration of 3.8 mmol/L to a human placental perfusion on three separate occasions during 14 h reduced the concentration of human chorionic gonadotropin by 75% (Boal *et al*., 1997). When human placental tissue and human trophoblast cells (Jar) were exposed to zidovudine at a concentration of 7.6 mmol/L for 48 h in culture and placental lobular tissue was perfused with 3.8 mmol/L zidovudine for 14 h, no zidovudine monophosphate was detected in any sample, and zidovudine inhibited cell proliferation by 72% in the Jar cells (Plessinger *et al*., 1997). [The Working Group noted that the concentrations used were 1000 times the plasma concentrations found normally after therapy.]

Zidovudine readily crosses the placenta of pregnant non-human primates, probably by passive diffusion (Lopez-Anaya *et al*., 1990). Zidovudine, zidovudine monophosphate and 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine glucuronide were detected in most fetal tissues after administration to pregnant rhesus monkeys (Patterson *et al*., 1997). After exposure of pregnant pigtailed macaques to zidovudine, the fetal:maternal plasma concentration ratio of zidovudine was 0.76 (Tuntland *et al*., 1998). Zidovudine administered to a baboon in late pregnancy resulted in a fetal:maternal plasma concentration ratio of 0.84, and the concentration of 3′-azido-3′-deoxy-5′-*O*-α-D-glucopyranosyl-thymidine in the fetus was seven times that of the mother (Garland *et al*., 1996; Stark *et al*., 1997; Garland *et al*., 1998).

4.2 Toxic effects

4.2.1 *Humans*

Zidovudine has a broad spectrum of toxicity, some of which may be difficult to distinguish from that due to the presence of the HIV virus in patients receiving anti-retroviral therapy (Styrt *et al*., 1996). Toxic effects caused only by the drug have been elucidated in uninfected individuals accidentally exposed to body fluids from HIV-infected patients, such as health-care workers given prophylactic zidovudine therapy, and in cases in which toxicity has resolved after discontinutation of therapy. In one study involving 674 health-care workers (Ippolito *et al*., 1997), 49% reported at least one adverse effect and 20% had discontinued treatment because of side-effects. Nausea was reported by 243 individuals, and others reported vomiting, gastric pain, asthenia and headache. Ten individuals had anaemia and seven had transient increases in the activity of liver enzymes. All of these effects were reversible when the drug was discontinued. This pattern of toxicity is similar in African–American, Hispanic and white HIV-positive patients (Jacobson *et al*., 1996).

(*a*) *Haematotoxicity*

Pancytopenia and bone-marrow aplasia are the dose-limiting toxic effects for zidovudine (Gill *et al*., 1987; Moore *et al*., 1996; Styrt *et al*., 1996). In early trials, doses of 1200 mg/day were not uncommon, and Moore *et al*. (1991b), who studied 886 patients with AIDS, reported that serious anaemia and neutropenia occurred in 30% and 37% of patients, respectively, who were given this dose. In the same study, 70% of patients had had to interrupt dosing at least once, and increased haematocrit was observed after dosing cessation in 52% of patients. In later trials at lower doses, the absolute rates of anaemia and neutropenia were considerably decreased: Ippolito *et al*. (1997) reported 10 cases of anaemia in 674 HIV-negative health-care workers, and Vella *et al*. (1994) reported that 23 of 936 patients with asymptomatic HIV infection had neutropenia. A related haematotoxic effect, macrocytosis with decreasing haemoglobin concentrations, was also observed in several studies (Mathé *et al*., 1996; Styrt *et al*., 1996; Vella *et al*., 1994). Vella *et al*. (1994) reported that 18 of 936 patients had a ≥ 25% decrease in haemoglobin concentration. A survey by the Spontaneous Reporting System of the US Food and Drug Administration (Styrt *et al*., 1996) included some 2000 case reports of toxicity due to zidovudine and confirmed the profile of haematotoxicity previously described in clinical trials.

(*b*) *Hepatotoxicity*

Transiently increased liver transaminase activities that returned to normal after discontinuation of the drug were reported in seven of 674 health-care workers (Ippolito *et al*., 1997) and in 63 of 936 HIV-positive patients (Vella *et al*., 1994). Hepatic toxicity appears to be a more frequent consequence of long-term (3–12 months) exposure to

zidovudine, and increases in liver enzyme activity have been accompanied by hepatomegaly with macrovesicular steatosis and frequently fatal lactic acidosis (Freiman *et al*., 1993; Olano *et al*., 1995; Acosta & Grimsley, 1999; Chariot *et al.*, 1999).

(*c*) *Myopathy and neurotoxicity*

Long-term use of zidovudine (2–12 months) has been associated with skeletal muscle and cardiomyopathy in adult patients with AIDS (Mhiri *et al.*, 1991; Peters *et al*., 1993; Dalakas *et al*., 1994; Cupler *et al*., 1995). Skeletal muscle myopathy has been observed in up to 27% of patients with a clinical presentation including fatigue, myalgia, muscle weakness, wasting, elevated serum creatine kinase activity and decreased carnitine concentration. In skeletal muscle biopsy samples, accumulation of lipid in muscle fibres, accumulation of mitochondria in the subsarcolemmal space (ragged red fibres) and morphologically abnormal mitochondria have been observed (Mhiri *et al*., 1991; Dalakas *et al*., 1994). In addition, long-term treatment with zidovudine has been associated with dilated cardiomyopathy in adult AIDS patients. Congestive heart failure, left ventricular dilatation, reduced ejection fractions (7–26%) and morphologically abnormal mitochondria have been demonstrated after prolonged (two years or more) use (Lewis, 1998). In one study (Barbaro *et al*., 1998), abnormal echocardiograms were observed in 76 of 962 HIV-positive patients. The clinical, morphological and biochemical manifestations of cardiac and skeletal muscle myopathy improved when zidovudine use was terminated (Mhiri *et al*., 1991; Lewis, 1998).

Analysis of neuromuscular function in a multicentre trial of patients receiving 600 mg/day zidovudine showed that 225 of 2467 patients had ≥ grade 2 peripheral neuropathy or distal symmetrical neuropathy. Of these cases, about 20% were considered to be related to treatment with zidovudine. Patients (7%) receiving zidovudine alone reported muscle weakness and ache, while 37% had difficulty in performing a series of tasks (Simpson *et al*., 1998).

4.2.2 *Experimental systems*

(*a*) *Haematotoxicity*

Most of the studies of the toxicity of zidovudine in animal models were performed in mice and, as in humans, the most frequently cited effect after either short-term or long-term dosing involved the haematopoietic system (Cronkite & Bullis, 1990; Bogliolo *et al*., 1991; Thompson *et al.*, 1991; Scheding *et al*., 1994; Omar *et al*., 1996; Inoue *et al*., 1997; Rao *et al*., 1998). Also as in humans, the effects were typically reversible within days to weeks after discontinuation of the drug. In one study (Du *et al*., 1992), normal bone marrow samples were obtained from patients undergoing hip replacement surgery and from mice, and the cells were exposed in culture to 0.1, 1.0, 10 or 100 μmol/L zidovudine. Continuous exposure caused greater inhibition of cell growth than a 1-h exposure, and the mouse cells were slightly more sensitive than the human cells to the toxic effects of zidovudine.

The effects of acute administration were examined by Scheding *et al*. (1994) in female B6C3F$_1$ mice given 30, 60, 90, 120 or 240 mg/kg bw zidovudine by bolus intravenous injection or by a 24-h intravenous infusion. The animals given bolus doses showed no toxicity, but those given the 24-h infusion had significantly decreased numbers of bone-marrow erythroid progenitor cells. Similar results were obtained by Bogliolo *et al*. (1991), who gave an intravenous dose of 240 mg/kg bw per day to mice for five days. The numbers of bone-marrow myeloid and erythroid progenitors reached the lowest point at five days and had returned to normal within two to five days after exposure. Exposure of mice to 500 mg/kg bw zidovudine for 7–14 days produced thymic involution, with a decreased percentage of CD4 and CD8 cells and a decrease in the ability of T cells to respond to foreign antigens (McKallip *et al*., 1995).

Long-term exposure to zidovudine results in more extensive haematopoeitic effects. For example, when 0.75 mg/mL zidovudine in drinking-water was administered to mice on days 84–687 of age, the primary toxic effects were thrombocytopenia and myelodysplasia (Inoue *et al*., 1997). In CBA/Ca mice given 1.0 mg/mL zidovudine in the drinking-water for seven weeks, neutropenia and lymphopenia, which did not resolve, and macrocytic anaemia and changes in bone-marrow cellularity were seen which returned to normal after discontinuation of treatment (Cronkite & Bullis, 1990). In other studies, doses of 25–1000 mg/kg bw zidovudine given to B6C3F$_1$ mice by gavage daily for 13 weeks caused bone-marrow depression and macrocytic anaemia, both of which were reversible when the drug was discontinued (Thompson *et al.*, 1991; Rao *et al*., 1998).

Cats infected with feline leukaemia virus were given 7.5, 15, 30 or 60 mg/kg bw zidovudine per day for 32–34 days (Haschek *et al*., 1990). The 60- and 30-mg doses produced anaemia by days 4 and 13, respectively, and decreased packed red cell volume, bone-marrow hypercellularity and splenic extramedullary haematopoiesis were seen, similar to the effects in humans.

Ayers *et al*. (1996b) studied the toxic effects of zidovudine in CD rats, beagle dogs and cynomolgus monkeys given large single doses and lower doses over longer periods. In rats given 225–250 mg/kg bw zidovudine twice daily and monkeys given 17.5–150 mg/kg bw twice daily for 3–12 months, macrocytic, normochromic anaemia was observed. After 12 months of dosing, the erythrocyte counts were decreased in rats and those of leukocytes were slightly decreased in monkeys. In dogs given 62.5–250 mg/kg bw zidovudine for 14 days, cell replication was inhibited in bone marrow, gastrointestinal epithelium and lymphoid tissue.

(*b*) *Myopathy*

Functional, morphological and biochemical changes, similar to the cardiac and skeletal muscle myopathy seen in HIV-positive individuals given long-term zidovudine treatment, have been observed in rats exposed to zidovudine (Lamperth *et al*., 1991; Pindado *et al*., 1994; McCurdy & Kennedy, 1996; Masini *et al.*, 1999). The animal models are particularly valuable because they clearly demonstrate that the drug

alone can induce muscle myopathy associated with mitochondrial damage (see Section 4.5), independently of the effects of the HIV virus. Lamperth *et al*. (1991) compared human muscle cultured with zidovudine for three weeks with muscle tissue from rats given 17–51 mg/kg bw zidovudine per day for three months. In the rats, zidovudine was preferentially concentrated in heart and skeletal muscle. In both species, the mitochondria were enlarged with disorganized or absent cristae and abnormal functioning of oxidative phosphorylation. Complexes I and II were observed. In a study (Pindado *et al*., 1994) in which rats were given 1–2 mg/mL zidovudine in the drinking-water for 30, 60 or 120 days, the cardiac muscle mitochondria were increased in size and showed disrupted cristae. McCurdy and Kennedy (1996) found decreased amounts of mitochondrial DNA and a diminished response to electrical stimulation in skeletal muscle of rats given 1 mg/mL zidovudine in the drinking-water for 35 days. They concluded that zidovudine treatment induces changes in mitochondria that result in diminished contractile capacity of skeletal muscle. Masini *et al.* (1999) demonstrated depleted concentrations of mitochondrial DNA in skeletal muscle of rats given 1 mg/mL zidovudine in the drinking-water (about 40 mg/kg bw zidovudine per day) for 90 days.

4.3 Reproductive and prenatal effects

4.3.1 *Humans*

Studies of the efficacy of zidovudine in reducing maternal–infant transmission of HIV have included information on the reproductive and prenatal effects of this drug.

In a review of the medical records of 104 HIV-infected women who were treated with zidovudine at various times during pregnancy at one clinic in India, there were eight spontaneous first-trimester abortions, eight therapeutic terminations and eight cases of fetal abnormality, of which two occurred in women treated during the first trimester (Kumar *et al.*, 1994). No specific abnormality could reasonably be attributed to the therapy.

White *et al.* (1997) used the files of an international Antiviral Pregnancy Register, set up in the USA in 1989 to monitor the safety of prenatal exposure to antiretroviral agents, to evaluate birth outcomes for 198 of 249 women who had been treated during pregnancy with zidovudine and for whom the pregnancy outcome was known by the authors. The outcomes included nine (4.5%) induced abortions, two (1.0%) spontaneous abortions and seven (3.7%; 95% CI, 1.6–7.8%) birth defects of which one occurred among the subgroup of 73 women who were exposed during the first trimester. The prevalence of birth defects among the zidovudine-treated pregnant women did not exceed the rate of 3–4% which is estimated to occur in the general population of the USA.

In a retrospective cohort study, Sperling *et al.* (1992) reported on 43 women with AIDS who had been treated at 17 different institutions in the USA, where they received

doses of zidovudine ranging from 300 to 1200 mg per day during part of or all of pregnancy. All 45 newborns, including two sets of twins, were born alive. No increased risk for premature births, intrauterine growth retardation or newborn asphyxia was found, and 41 of the 45 infants were born at term. Among the 12 newborns who had been exposed to zidovudine during the first trimester, no malformations were reported.

In a randomized, placebo-controlled trial, Connor *et al*. (1994) included 477 HIV-infected pregnant women from various treatment centres in France and the USA in 1991–93. The women were randomly assigned to receive either zidovudine or placebo. During the study period, 409 gave birth to 415 liveborn infants. There were eight fetal or neonatal deaths, five in the group receiving zidovudine and three in the group given the placebo. None of these deaths was considered by the authors to be attributable to the drug. Seven infants died beyond the neonatal period, six (two in the group given zidovudine) from HIV infection and one (in the group given zidovudine) due to trauma.

In order to determine the safety of zidovudine administered during pregnancy, Sperling *et al*. (1998) reanalysed the findings of a randomized placebo-controlled trial in which HIV-infected women between 14 and 34 weeks' gestation with CD4 cell counts > 200/μL and no maternal indications for antiretroviral therapy were enrolled from 55 treatment centres in France and the USA. During the inclusion period of 1991–93, 424 eligible women were randomized to either zidovudine or placebo, and the women were followed through six months *post partum*, while their infants were followed through 18 months of age. Five women given zidovudine and two given placebo had either a spontaneous abortion or a stillbirth. Among the infants born live to women receiving zidovudine, 19 (9%) had major structural abnormalities and 28 (13%) had minor structural abnormalities, while among the infants born live to women given placebo the equivalent figures were 17 (8%) and 35 (17%).

Lorenzi *et al*. (1998) reported on a small prospective study conducted in Switzerland of 33 HIV-infected women who had received combined antiretroviral therapy including zidovudine during pregnancy in 1996–98. Among the 33 liveborn neonates, one (3.0%) case of congenital malformation was found.

In the French National Epidemiological Network, records have been kept of 1754 children of women seropositive for HIV-1 who were treated during pregnancy with zidovudine (500 mg per day) or with zidovudine and lamivudine (300 mg per day) for a mean prenatal exposure of 17.2 weeks (range, 0–40 weeks). After birth, the children of these women were given daily doses of 8 mg/kg bw zidovudine and/or 4 mg/kg bw lamivudine for an average of 5.2 weeks (range, 2–6 weeks). Two of the HIV-negative children, who had received both zidovudine and lamivudine transplacentally, died at approximately one year of age with symptoms of mitochondrial disorders, including seizures, brain lesions identified by nuclear magnetic resonance, persistent lactic acidosis and abnormal mitochondrial oxidative phosphorylation (respiratory chain) function in skeletal muscle, lymphocytes and liver. Six other HIV-negative children between the ages of seven months and 4.4 years, two of whom had received zidovudine

and lamivudine and four of whom had received zidovudine alone, had various combinations of brain lesions, seizures and persistent lactic acidosis. All of these children had abnormal mitochondrial oxidative phosphorylation, and three had no other clinical symptoms (Blanche *et al.*, 1999).

Culnane *et al.* (1999) reported on the Pediatric AIDS Clinical Trial Group Protocol 076, a multicentre, randomized, double-blind, placebo-controlled trial in which zidovudine was used to prevent perinatal HIV-1 transmission. The median age of 234 uninfected children born to 2330 HIV-infected women at the time of the follow-up visit was 4.2 years (range, 3.2–5.6 years). There were no significant differences between children exposed to zidovudine (122) and those who received placebo (112) with respect to weight, height, head circumference or cognitive development. No deaths or malignancies were reported.

4.3.2 *Experimental systems*

Bone-marrow aspirates from women of child-bearing age, bone marrow and liver from seven mid-trimester abortuses and umbilical cord blood from seven term infants were treated *in vitro* with zidovudine at a concentration of 0.1, 1.0, 10, 100 or 500 µmol/L. Erythroid progenitor cells from all fetal and neonatal sources were more sensitive to zidovudine than those from the bone marrow of adult women (Shah *et al.*, 1996).

Aliquots of 16–20 fertilized mouse (FVB/N) oocytes at the one-cell stage were isolated and treated with zidovudine at a concentration of 0, 0.1, 1.0 or 10 µg/mL. Exposure to the two higher doses was reported to reduce blastocyst formation (Toltzis *et al.*, 1991). This study was replicated with two-cell embryos in the same laboratory, with the same result (Toltzis *et al.*, 1994).

Male and female embryos collected from pregnant Wistar rats on day 9.5 of gestation were cultured for 48 h with 0, 50, 500, 1000 or 3000 µmol/L zidovudine. At the highest concentration, zidovudine was reported to produce a 40% incidence of abnormal embryos (Klug *et al.*, 1991).

In a 14-week study of reproductive toxicity in 10 $B6C3F_1$ mice dosed by gavage with 0, 100, 800 or 2000 mg/kg bw zidovudine, no treatment-related effects were found on spermatid or epididymal spermatozoal parameters in males or oestrone cycle characteristics in females (National Toxicology Program, 1999).

The toxicity of zidovudine was evaluated in groups of 10 adult male Swiss (CD-1) mice treated by gavage with 200 or 400 mg/kg bw zidovudine or with vehicle for 20–21 days and 20 adult female mice given the same doses for either 28–32 days or on days 6–15 of gestation. Zidovudine treatment at both doses caused decreased sperm motility and reduced the number of pregnant mice per group. In pregnant dams, the average numbers of corpora lutea and implantations per litter were not affected by treatment, but the number of live fetuses per litter was decreased and the average number of early and/or late deaths increased. The mean fetal body weight per litter

was reduced by treatment with either dose (National Institute of Environmental Health Sciences, 1998).

In a separate study of the same design, zidovudine at doses of 100, 200 or 400 mg/kg bw given to pregnant mice decreased body-weight gain, reportedly due to reduced litter sizes, increased the number of resorptions and reduced the fetal weights per litter. No statistically significant increase in the number of litters or fetuses with gross external alterations was reported, and no statistically significant effect on sperm motility was observed (National Institute of Environmental Health Sciences, 1999).

Pregnant transgenic mice carrying the Moloney murine leukaemia virus in the germ line were treated with zidovudine dissolved in the drinking-water at a concentration of 0.1, 0.2, 0.4 or 0.6 mg/mL. Dosing began on either gestational day 10 or 19 and was continued throughout lactation and weaning. After weaning, the offspring received zidovudine directly in their drinking-water. No results were given for the group receiving 0.6 mg/mL zidovudine, but at the other doses, only mild macrocytic anaemia was reported. No gross teratological effects and no effect on litter size were reported. Histopathological analyses of the offspring revealed no specific sequelae (Sharpe *et al*., 1988). [The Working Group noted that the methods for collection of reproductive and histopathological data were not described and no individual or mean data were shown for these developmental toxicological end-points.].

Mature, female C3H/He mice were treated with 0, 0.25, 0.50 or 2.5 mg/mL zidovudine in the drinking-water at eight weeks of age and were mated after eight weeks of therapy. Dosing was continued throughout gestation. Two of the three untreated mice but none of the nine treated animals produced offspring. Mature female C3H/He mice (20 per group) were either untreated or treated with 0.25 mg/mL zidovudine in the drinking-water beginning at six weeks of age and were mated after six weeks of treatment. Dosing was continued up to days 12–17 of gestation. Treatment with zidovudine was reported to reduce the occurrence of pregnancy and the number of fetuses per litter and to increase the number of resorptions. No physical anomalies were noted on gross examination of surgically removed fetuses at 12–17 days of gestation (Toltzis *et al*., 1991). [The Working Group noted that the gestational days of exposure and age at sacrifice were not clear, having an apparent range of 12–17 days.]

Groups of 6–10 pregnant CD-1 mice were treated with zidovudine at 0, 0.1 or 0.5 mg/mL in the drinking-water on days 1–13 of gestation. Fetuses, examined at day 13 of gestation, were reported to be smaller, with fewer fetuses per litter and decreased colony-forming ability of erythroid progenitor cells collected from fetal hepatic tissue (Gogu *et al*., 1992).

Groups of 7–12 pregnant Swiss-derived CD-1 mice were given 0, 0.2, 0.4 and 2.0 mg/mL zidovudine in the drinking-water from day 10 of gestation to delivery. The body weights (day 16) and water intake (days 12–14 and 14–16) of the dams at the highest dose were significantly decreased. Only two of 12 dams at this dose had viable litters, and the pups in these litters were lighter, smaller and less active than controls. Maternal cannibalization may have accounted for some of the pup deaths. The serum

concentrations of zidovudine in treated dams were 0.87 μg/mL at 0.2 mg/mL, 1.4 μg/mL at 0.4 mg/mL and 1.7 μg/mL at 2.0 mg/mL. Behavioural analysis of the pups of dams given 0.2 or 0.4 mg/mL and comparison with controls revealed reduced pup weight gain (males and females combined), delayed appearance of the pole-grasping reflex (males at 0.4 mg/mL only) and slight but significant impairment during acquisition sessions of the passive avoidance test (at 0.2 and 0.4 mg/mL). The dose of 0.4 mg/mL appeared to reduce the sex differences in two sexually dimorphic aspects of the social behaviour repertoire (Calamandrei *et al*., 1999).

Groups of six male Wistar rats, 40 days old, were treated with 0, 0.1 or 1.0 mg/mL zidovudine (approximately equivalent to 15 and 150 mg/kg bw per day) in the drinking-water for four weeks. Body-weight gain was similar in control and treated animals, but ventral prostate weight, seminal vesicle weight and serum testosterone concentrations were decreased and serum luteinizing hormone and prolactin concentrations were increased as compared with controls (Sikka *et al*., 1991).

Groups of 20 pregnant Wistar rats were treated with water or 100 mg/kg bw zidovudine orally three times at 5-h intervals (total dose, 300 mg/kg bw) on day 10 of gestation. No adverse effects were reported on maternal body weight, food consumption, reproductive capacity or haematological parameters. No effects of zidovudine on the survival or growth of offspring and no treatment-related gross histopathological lesions were reported in the weanling rats. The mean concentration of zidovudine in day-10 embryonal homogenates, collected 30 min after the third dose, was 21 μg/g tissue (Greene *et al*., 1990).

Groups of 35 male and female Sprague-Dawley CD rats were treated twice daily by gavage with zidovudine in 0.5% methylcellulose at a dose of 0, 25, 75 or 225 mg/kg bw. Starting at seven weeks of age, F_0 generation males were dosed for 85 days before mating and afterwards, for a total of 175 days. F_0 females were treated daily for 26 days before mating and throughout gestation and lactation. No consistent adverse effects were reported in the males or in the offspring of untreated females. Early embryo mortality was increased and the number of live fetuses per litter was decreased at 75 and 225 mg/kg bw zidovudine. Fetal body weight was decreased at the highest dose (Greene *et al*., 1996).

Groups of 12 mature female CD rats were treated twice daily by gavage with zidovudine in 0.5% methylcellulose at a dose of 225 mg/kg bw 26 days before mating through postnatal day 15; 26 days before mating through to day 10 of gestation; 26 days before mating until pregnancy was confirmed or from day 1 of gestation through postnatal day 15. Zidovudine-induced embryotoxicity was limited to early embryos, and no gross or histological changes were reported in the offspring (Greene *et al*., 1996).

Groups of 21–24 pregnant CD rats were treated orally twice daily with zidovudine at a dose of 0, 62.5, 125 or 250 mg/kg bw on days 6–15 of gestation. No toxic effects were reported in dams or their offspring (Greene *et al*., 1996).

Groups of 30 pregnant CD rats were treated twice daily by gavage with zidovudine in 0.5% methylcellulose at a dose of 0, 25, 75 or 225 mg/kg bw from day 17

of gestation through delivery until postnatal day 21. No consistent or dose-related effects on growth, development (including behaviour) or reproductive performance were reported (Greene *et al*., 1996).

Sixteen pregnant VAF Sprague-Dawley rats were treated by gavage with 150 mg/kg bw per day zidovudine for 22 days from day 1 of gestation. A control group of 14 rats received water. On day 22 of gestation, no gross structural malformations were found in the 12 litters examined (six per group). Treatment reduced the litter size and increased the weights of both male and female offspring. On postnatal days 21–22, the pups were injected subcutaneously with amphetamines (0.25–1.0 mg/kg bw) and their behaviour was observed. The locomotion response to amphetamines was increased only in female pups given zidovudine (Applewhite-Black *et al*., 1998).

Pregnant Sprague-Dawley rats were given zidovudine by gavage in water at a dose of 0, 50, 100 or 150 mg/kg bw on days 19–22 of gestation, and individual offspring were treated by gavage on postnatal days 2–20 at the same doses. Behaviour after intraperitoneal injection of amphetamines (0.25, 0.50, 0.75 or 1.0 mg/kg bw) was assessed on postnatal day 21. The authors concluded that perinatal exposure to zidovudine altered one aspect of behaviour—locomotion—the threshold for this effect depending on sex. Because no effects were seen on litter size or on maternal or pup weight gain, they concluded that zidovudine alters neurodevelopmental processes without producing overt toxicity (Busidan & Dow-Edwards, 1999a). In a follow-up study of 12–15 litters per group, behaviour after an intraperitoneal injection of amphetamine (0.1, 0.5, 1.0 or 2.0 mg/kg bw) was assessed on postnatal days 59–65. Treatment did not alter open-field behaviour (Busidan & Dow-Edwards, 1999b).

Pregnant New Zealand white rabbits were treated orally twice daily at a 6-h interval with zidovudine in 0.5% methylcellulose at a dose of 0, 37.5, 75 or 250 mg/kg bw on days 6–18 of gestation. Twenty-two rabbits per group were killed on day 29. Dams given 250 mg/kg bw were reported to have reduced weight gain during days 6–18 of gestation and decreased haemoglobin concentration, haematocrit and red blood cell count. The effects on fetuses included increased numbers of resorptions and decreased weights at 250 mg/kg bw. No teratogenic effects were reported. At 250 mg/kg bw, the mean peak concentration of zidovudine in maternal plasma was 92.7 μg/mL (Greene *et al*., 1996).

Female pigtail monkeys (*Macaca nemestrina*) were given zidovudine at a dose of 1.5 mg/kg bw or vehicle every 4 h via a gastric catheter for at least 10 days before conception and throughout pregnancy. Nine of the zidovudine-treated monkeys became pregnant and carried to term, as did seven control monkeys. The treated mothers developed asymptomatic macrocytic anaemia and showed decreased total leukocyte counts. The zidovudine-exposed infants were mildly anaemic at birth and showed deficits in growth, rooting and snouting reflexes and in the ability to fixate and follow near stimuli visually. These deficits disappeared over time after birth (Ha *et al*., 1994, 1998).

4.4 Genetic and related effects

4.4.1 *Humans*

There are few published data of the effects of zidovudine on the genome of zidovudine-treated patients. The drug has not been reported to be mutagenic. Shafik *et al.* (1991) reported high levels of clastogenicity, but a later study with more reliable methods failed to find a similar effect (Witt *et al.*, 1999, abstract). Shafik *et al.* (1991) assessed the frequency of chromosomal aberrations in HIV-infected patients who were non-smokers and who had received zidovudine at 1200 mg/day for four weeks to seven months. One hundred metaphases from first-division cells from each culture of peripheral lymphocytes were scored for aberrations, mainly of the chromatid type. The frequencies of breaks in the zidovudine-treated and non-HIV-infected control groups were 8.3 ± 2.0 and 0.5 ± 0.3 per 100 cells, respectively. Witt *et al.* (1999, abstract) evaluated the frequency of chromosomal aberrations in two healthy men occupationally exposed to HIV and in 22 HIV-positive men who had not previously received antiviral drugs, after treatment with zidovudine and other dideoxynucleosides according to standard protocols. The mitotic index and frequency of chromosomal aberrations were measured in peripheral lymphocytes collected before initiation of drug therapy and at approximately four and 12 weeks during treatment. No significant treatment-related changes in mitotic index or in the percentage of aberrant cells were observed.

Olivero *et al.* (1999) quantified the zidovudine incorporated into peripheral blood leukocyte DNA from 24 of 28 HIV-positive non-pregnant adults receiving 600 mg zidovudine per day and in peripheral blood leukocyte DNA from eight of 12 pregnant women who had received zidovudine for periods varying from the last three weeks to the whole nine months of pregnancy. The values varied from 22 to 544 molecules of zidovudine/10^6 nucleotides. Of a group of 22 infants exposed to zidovudine *in utero*, including children of the 12 women mentioned above, 15 had measurable values for incorporation of zidovudine into DNA in cord blood leukocytes, varying from 22 to 452 molecules of zidovudine/10^6 nucleotides. The results show that the amounts of zidovudine incorporated into human DNA are similar in adults and in infants exposed *in utero*.

4.4.2 *Experimental systems*

These studies are summarized in Table 1.

(*a*) *In vitro*

Testing of zidovudine in prokaryotic systems *in vitro* is limited to a few studies performed by three groups. Zidovudine induced reverse mutation in only one strain of *Salmonella typhimurium* and produced marginal or no differential toxicity in *Escherichia coli* and *Bacillus subtilis*. Mamber *et al*. (1990) assessed the ability of zidovudine and representative dideoxynucleosides to induce two SOS functions, cell filamentation

Table 1. Genetic and related effects of zidovudine

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Escherichia coli BR513 (*uvr*B *env*A *lacZ::lambda*), prophage induction, SOS repair (spot and liquid suspension tests)	+	NT	0.064	Mamber *et al*. (1990)
Escherichia coli PQ37 (*uvr*A *rfa lacZ::sul*A), cell filamentation, SOS repair (spot and liquid suspension tests)	+	NT	0.5	Mamber *et al*. (1990)
Escherichia coli CM871 (*uvr*A *rec*A *lex*A), differential toxicity (vs *Escherichia coli* WP2)	(+)	NT	1000	Mamber *et al*. (1990)
Bacillus subtilis M45 *rec* strain, differential toxicity	–	NT	NR	Mamber et al. (1990)
Salmonella typhimurium TA100, TA1535, TA1537, TA1538, TA88, reverse mutation	–	–	10 µg/plate	Ayers *et al*. (1996a)
Salmonella typhimurium TA100, TA104, TA 1535, TA98, TA97, reverse mutation	–	–	1–3 µg/plate	National Toxicology Program (1999)
Salmonella typhimurium TA 102, reverse mutation	(+)	(+)	0.3 µg/plate	National Toxicology Program (1999)
Bacilllus subtilis H17, gene mutation	–	NT	NR	Mamber *et al*. (1990)
Gene mutation, Chinese hamster ovary cells *in vitro*, *Hprt* locus	–	NT	10 000	Grdina *et al*. (1992)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus, 4-h treatment *in vitro*	(+)[c]	(+)	1000	Ayers *et al*. (1996a)
Gene mutation, mouse lymphoma L5178Y cells, *Tk* locus, 24-h treatment *in vitro*	+	NT	25	Ayers *et al*. (1996a)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	NT	500	González Cid & Larripa (1994)
Sister chromatid exchange, Chinese hamster cells *in vitro*	+	+	8.3	National Toxicology Program (1999)
Chromosomal aberrations, Chinese hamster cells *in vitro*	+	NT	1000	González Cid & Larripa (1994)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Chromosomal aberrations, Chinese hamster cells *in vitro*	–	–	2500	National Toxicology Program (1999)
Cell transformation, BALB/c 3T3 mouse cells	+	NT	0.5	Ayers *et al*. (1996a)
Gene mutation, human HepG2 cells *in vitro*, *HPRT* locus	+	NT	100	Grdina *et al*. (1992)
Gene mutation, human TK6 lymphoblastoid cells *in vitro*, *TK* locus	+	NT	9 × 3 d	Meng *et al*. (2000a)
Gene mutation, human TK6 lymphoblastoid cells *in vitro*, *HPRT* locus	+	NT	80 × 3 d	Sussman *et al*. (1999)
Sister chromatid exchange, human lymphocytes *in vitro*	+	NT	50	González Cid & Larripa (1994)
Micronucleus formation, human lymphocytes *in vitro*	+	NT	500	González Cid & Larripa (1994)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	100	González Cid & Larripa (1994)
Chromosomal aberrations, human lymphocytes *in vitro*	+	NT	3	Ayers *et al*. (1996a)
Chromosomal aberrations, H9 human lymphocytic cells *in vitro*	+	NT	6.7 × 7 mo	Agarwal & Olivero (1997)
Irreversible telomere shortening, mouse brain and liver cells *in utero*	+		25 mg/d, d 12–18 of gestation	Olivero *et al*. (1997)
Irreversible telomere shortening, *Erythrocebus patas* cells *in utero* (various organs)	–		10 mg/d, 5 d/wk; last 9.5–10 wk of gestation	Olivero *et al*. (1997)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Micronucleus formation, mouse peripheral blood lymphocytes and bone-marrow cells *in vivo*	+		100 po × 4 wk	Oleson & Getman (1990); Ayers *et al*. (1996a)
Micronucleus formation, mouse peripheral blood lymphocytes and bone-marrow cells *in vivo*	+		NR iv × 4 d	Oleson & Getman (1990); Ayers *et al*. (1996a)
Micronucleus formation, B6C3F$_1$ mouse bone-marrow cells *in vivo*	+		200 po × 3	Phillips *et al*. (1991)
Micronucleus formation, B6C3F$_1$ mouse bone-marrow cells *in vivo*	+		100 po × 13 wk	Phillips *et al*. (1991)
Micronucleus formation, Swiss Webster mouse bone-marrow cells *in vivo*	–		14.4 ip × 1	Motimaya *et al*. (1994a)
Micronucleus formation, Swiss Webster mouse bone-marrow cells *in vivo*	–		28.6 ip × 1	Motimaya *et al*. (1994b)
Micronucleus formation, BALB/c mouse bone-marrow cells *in vivo*	+		17 ip × 5; 2 wk	Dertinger *et al*. (1996)
Micronucleus formation, rat bone-marrow cells *in vivo*	+		500 po × 7	Oleson & Getman (1990); Ayers *et al*. (1996a)
Chromosomal aberrations, mouse bone-marrow cells *in vivo*	+		33 po (drinking-water) × 4 wk	Olivero *et al*. (1994a)
Chromosomal aberrations, rat bone-marrow cells *in vivo*	–		300 iv × 1	Ayers *et al*. (1996a)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Binding (covalent) to telomeric and Z-DNA, Chinese hamster ovary cells *in vitro*	+		214	Olivero & Poirier (1993); Gomez *et al*. (1995); Parra *et al*. (1997)
Binding (covalent) to DNA, Chinese hamster ovary cells *in vitro*	+		5.3	Olivero *et al*. (1994b)
Binding (covalent) to DNA, mouse NIH 3T3 cells *in vitro*	+		5.3	Olivero *et al*. (1994b)
Binding (covalent) to DNA, CCRF/CEM human lymphoid cells *in vitro*	+		0.27	Avramis *et al*. (1989)
Binding (covalent) to DNA, human bone-marrow cells *in vitro*	+		2.7	Sommadossi *et al*. (1989)
Binding (covalent) to DNA, K562 human leukaemic cells *in vitro*	+		2.7	Vazquez-Padua *et al*. (1990)
Binding (covalent) to DNA, HL60 human leukaemic cells *in vitro*	+		5.3	Olivero *et al*. (1994b)
Binding (covalent) to DNA, HCT-8 human colon cancer cells *in vitro*	+		5.3	Darnowski & Goulette (1994)
Binding (covalent) to DNA, H9 human lymphocytic cells *in vitro*	+		6.7 × 7 mo	Agarwal & Olivero (1997)
Binding (covalent) to DNA, TK6 human lymphoblastoid cells *in vitro*	+		80 × 3 d	Sussman *et al*. (1999)
Binding (covalent) to DNA, TK6 human lymphoblastoid cells *in vitro*	+		9 × 3 d	Meng *et al*. (2000b)
Binding (covalent) to DNA, mouse vaginal epithelial cells *in vivo*	+		33 po (drinking-water) × 4 wk	Olivero *et al*. (1994a)
Binding (covalent) to nuclear and mitochondrial DNA, mouse kidney, liver, lung and skin cells *in utero*	+		25 mg/d; d 12–18 of gestation	Olivero *et al*. (1997)

Table 1 (contd)

Test system	Result[a]		Dose[b] (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Binding (covalent) to nuclear and mitochondrial DNA, *Erythrocebus patas* cells *in utero* (brain, heart, kidney, liver, lungs, placenta)	+		10 mg/d, 5 d/wk; last 9.5–10 wk of gestation	Olivero *et al*. (1997)

[a] +, positive; (+), weak positive; –, negative; NT, not tested
[b] LED, lowest effective dose; HID, highest ineffective dose; in-vitro tests, μg/mL; in-vivo tests, mg/kg bw per day; NR, not reported; po, oral; iv, intravenous; ip, intraperitoneal; mo, month; d, day; wk, week
[c] Positive at 4000–5000 μg/mL only

and prophage lambda, in *E. coli*. Zidovudine was the most potent inducer of the SOS response, with a minimal active concentration that was 100-fold lower than those of the dideoxypurine nucleosides. These results indicate that zidovudine and dideoxynucleosides do not cause DNA lesions that are removed by the excision repair (*uvr*A) or error-free postreplication repair (*rec*A) processes. Rather, in acting as DNA chain terminators, they may generate an SOS-inducing response leading to inhibition of DNA replication.

The results of studies in animal cells *in vitro* have generally indicated mutagenic effects of zidovudine. In several tests with high doses of zidovudine that are not clinically relevant, it did not induce mutations at the hypoxanthine-guanine phosphoribosyl (*Hprt*) locus but significantly increased the frequencies of sister chromatid exchange and chromosomal aberrations in cultured Chinese hamster ovary cells. Zidovudine was also mutagenic at the thymidine kinase (*Tk*) locus of mouse lymphoma cells, and it caused cell transformation in 3T3 mouse cells.

The results of tests for gene mutation and clastogenicity in zidovudine-exposed human cells have been consistently positive, both at high doses and at low doses approximating the plasma concentrations in treated patients. Zidovudine induced mutations at the *HPRT* locus of HepG2 cells at high doses, but addition of a cytoprotective agent concomitantly or after zidovudine was effective in reducing the mutagenic effects. Sussman *et al*. (1999) investigated the relationships between incorporation of zidovudine into DNA [see (c) below], mutant frequency and the spectrum of deletion mutations in *HPRT* of human lymphoblastoid TK6 cells exposed to a concentration 10–20 times higher than the peak plasma concentrations in adult patients given a dose of 200 mg zidovudine. Treatment of TK6 cells with zidovudine significantly increased the *HPRT* mutant frequency, and molecular analyses showed that the differences between the control and treated groups was due mainly to an increase (by 16–37%) in the frequency of total gene deletion mutations. These results indicate that the primary mechanism of zidovudine-induced mutagenicity in TK6 cells is production of large deletions as a result of incorporation into DNA and subsequent chain termination. The mutational spectra also suggested that zidovudine induces point mutations, either directly by some unknown mechanism or indirectly by damaging genes that affect the frequency of endogenous mutational events. This hypothesis is supported by the finding of distinctive point mutations in Ha-*ras* in skin tumours from mice exposed *in utero* to zidovudine.

Sussman *et al*. (1999) also exposed TK6 cells to a minor metabolite of zidovudine, 3′-amino-3′-deoxythymidine, to evaluate its relative cytotoxic and mutagenic potency. 3′-Amino-3′-deoxythymidine was more cytotoxic than zidovudine at equimolar doses, and the amino metabolite did not produce a mutagenic response in TK6 cells. 3′-Amino-3′-deoxythymidine thus appears to contribute little to the mutagenic potency of zidovudine *in vivo* as only small amounts are formed in humans and relatively small amounts are incorporated into DNA after zidovudine treatment of human cells (Darnowski & Goulette, 1994; Acosta *et al*., 1996).

Meng *et al*. (2000a) evaluated the relationships between incorporation of zidovudine into DNA, mutant frequency and loss of heterozygosity at the *TK* locus of TK6 cells exposed to zidovudine at concentrations down to the peak plasma levels found in some patients. The *TK* mutant frequencies increased in a time-dependent manner in treated cells and appeared to approach a plateau after 6 days of exposure. The frequencies were significantly increased over the background value at all doses, including that which corresponded to a clinically relevant concentration. Southern blot analyses indicated that 84% of the zidovudine-induced *TK* mutants had loss of heterozygosity due to large deletions, consistent with the known mechanism of action of zidovudine as a DNA chain terminator.

The fact that TK6 cells are heterozygous at the *TK* locus and hemizygous at the *HPRT* locus can affect both the magnitude and the nature of the mutagenic response to chemically induced lesions at these two reporter genes (Meng *et al*., 2000b). Many mutagenic mechanisms that involve homologous interaction, such as gene conversion and mitotic recombination, cannot occur at the X-linked *HPRT* locus. In addition, multilocus deletions are likely to be lethal to the *HPRT* gene, because these gross deletions may span the adjacent genes essential for cell survival. Therefore, if the primary mechanism of mutation induction by a chemical implicates homologous interaction or a large deletion, the mutagenicity of that agent will be significantly underestimated in the *HPRT* assay; indeed, the average zidovudine-induced *TK* mutant frequency was significantly greater than the average zidovudine-induced *HPRT* mutant frequency in the same cell samples. Awareness of these differences is important because *HPRT*, but not *TK*, mutant frequencies can be measured in zidovudine-treated patients (Meng *et al*., 2000b).

The potential role of large-scale DNA damage in zidovudine-induced mutagenicity is supported by the finding of increased frequencies of sister chromatid exchange, micronuclei and chromosomal aberrations in zidovudine-exposed human cells *in vitro*. Agarwal and Olivero (1997) reported that seven months' exposure of human lymphocytic H_9 cells in culture to concentrations of zidovudine equivalent to the peak plasma concentrations of this drug in some patients significantly increased the frequency of chromosomal aberrations, the most dramatic increases being observed in the number of breaks and fragments. These two aberrations are consistent with integration of zidovudine into DNA and prevention of chain elongation.

Gomez *et al*. (1998) sought to determine if long-term exposure of human cells to zidovudine *in vitro* results in telomere shortening and if this shortening is reversible. They cultured HeLa cells with zidovudine and found that, as the passage number increased, the length of the telomere decreased markedly. This phenomenon correlated with incorporation of zidovudine into the telomere by telomerase and subsequent chain termination. The shortened telomeric repeats did not elongate after being cultured without zidovudine for additional passages, but no evidence of cell senescence was detected.

(*b*) *In vivo*

Studies of the mutagenicity of zidovudine in animals *in vivo* are limited to assays for micronuclei and chromosomal aberrations in rodents; the results of seven of 10 studies were positive. In the only study of micronucleus formation in which negative results were obtained, Motimaya *et al*. (1994a,b) reported no effect in mice given single intraperitoneal doses of 14.3–28.6 mg/kg bw. Although the doses used approximated the recommended daily dose for a person of average (70 kg) weight, Shelby (1994) pointed out that zidovudine therapy in patients involves long-term treatment. The finding raised questions about the clastogenic potential of zidovudine at clinically relevant doses as well as the sensitivity of the micronucleus assays used: either low doses of zidovudine do not induce micronuclei in mice or the genotoxic effects at these doses are too small to be detected in the tests as performed (Shelby, 1994). Dertinger *et al*. (1996) addressed these issues by scoring micronuclei with high throughput flow cytometry after intraperitoneal administration of zidovudine at 0 or 17 mg/kg bw to groups of five female and five male mice on five days a week for two weeks. The modest yet highly significant clastogenic effect observed in both sexes was below the limit of detection of conventional micronucleus assays which rely on microscopic inspection to score the rare micronucleated cell population. This study strongly suggested that low, clinically relevant concentrations of zidovudine are clastogenic.

Olivero *et al*. (1997) reported that transplacental exposure of mice to zidovudine at 25 mg/day by gavage during the last third of gestation resulted in shorter chromosomal telomeres in the liver and brain of most newborn mice. This effect was not observed in offspring of *Erythrocebus patas* monkeys given 20% of the human equivalent dose of zidovudine during the last half of gestation.

(*c*) *DNA incorporation of zidovudine*

The direct effects of zidovudine on DNA include incorporation into host cell nuclear and/or mitochondrial DNA and/or inhibition of nuclear or mitochondrial DNA polymerases. The first phenomenon results in termination of the DNA chain, and both phenomena lead to a reduction of DNA synthesis, with resultant effects on cell growth and viability. Thus, techniques have been devised to determine the amount of zidovudine incorporated, to elucidate the conditions under which such incorporation occurs and to define the generalized toxic and specific mutagenic consequences of such incorporation. The observed toxic and mutagenic effects of zidovudine may also be related to indirect interaction with host cell DNA through zidovudine-mediated alterations in deoxynucleotide pools, inhibition of DNA anabolic enzymes and interference with DNA repair processes. Only a brief review of the experimental evidence for incorporation of zidovudine into DNA as it relates to possible mutagenic and tumorigenic outcomes is given here.

Numerous studies have demonstrated that zidovudine is incorporated into the nuclear DNA of a variety of cell types (e.g. ovary cells, fibroblasts, lymphoblastoid/

lymphocytic cells, leukaemic cells, colon cancer epithelium and vaginal epithelium) *in vitro* and *in vivo* and in whole tissues in several species (e.g. hamsters, mice, monkeys and humans). As mentioned earlier, zidovudine is preferentially incorporated into the telomeric regions of some cell types (Olivero & Poirier, 1993; Gomez *et al*., 1995; Parra *et al*., 1997).

In most of the studies referred to in Table 1, mutagenicity and DNA incorporation were not conducted in the same samples, precluding a direct evaluation of the relationship between the two. In the study of Meng *et al*. (2000a) of the relationship between duration of exposure and concentration, incorporation of zidovudine into genomic DNA and mutagenic responses at the *HPRT* and *TK* loci of TK6 cells exposed in culture to concentrations down to the peak plasma concentrations in some patients, they found a significant correlation between incorporation of zidovudine into DNA and *HPRT* and *TK* mutant frequencies in relation to both duration and dose, strongly indicating that incorporation of zidovudine into nuclear DNA has a direct role in its mutagenicity.

In the study of Sussman *et al*. (1999), described above, of the relationships between duration of exposure, incorporation of zidovudine into genomic DNA and the mutagenic response at the *HPRT* locus of TK6 cells exposed in culture, although the concentration of zidovudine in the medium was 10–20 times higher than the peak plasma concentrations in some adult patients, the amount of zidovudine incorporated into DNA was comparable to those found after exposure of CD-1 mice, *E. patas* and *Macaca mulatta* monkeys and human infants to zidovudine *in utero*. Furthermore, exposure of TK6 cells to the equivalent of peak plasma concentrations of zidovudine for three days led to the incorporation of much smaller amounts of zidovudine than in cells from monkey and human infants exposed to clinical doses of zidovudine *in utero* (Meng *et al*., 2000a). Comparisons of the amounts of zidovudine incorporated into DNA with duration and dose are presented in Table 2.

It is noteworthy that short-term exposure (three days) of human cells in culture to moderately high concentrations of zidovudine, short-term transplacental exposure of mice to high concentrations and long-term transplacental exposure of monkeys and humans to clinical doses of zidovudine had nearly the same effect in terms of incorporation into DNA. Exposure of human cells in culture to zidovudine can be correlated directly with a significant mutagenic response, and the exposure of mice led to increased incidences of transplacentally induced cancers and inactivation of Ha-*ras* oncogene in skin tumours. These results indicate that the dose and plasma concentrations of zidovudine do not account by themselves for its mutagenic and carcinogenic effects. Rather, the duration of exposure and other yet to be determined factors (such as species and inter-individual genetic variations in enzymes involved in anabolism of zidovudine and repair of zidovudine-induced DNA damage) must participate in zidovudine-mediated mutagenesis and carcinogenesis.

Table 2. Relationships between exposure to zidovudine, incorporation into DNA and mutagenic or carcinogenic effects

Sample source	Cell or tissue type	Dose of zidovudine or exposure	Molecules of zidovudine per 10^6 nucleotides	Mutagenic or carcinogenic response	Reference
Pregnant women	Peripheral blood lymphocytes	600 mg/day orally during last three weeks up to nine months of pregnancy	25–215	Unknown	Olivero *et al.* (1999)
Newborns	Cord blood lymphocytes	Transplacental via their mothers' therapy	22–452	Unknown	Olivero *et al.* (1999)
Monkey fetuses (*Macaca mulatta*)	Brain, heart, liver, lung, skeletal muscle, placenta, testis	In pregnant monkeys: 8 mg/kg bw intravenously 4 h before hysterectomy	29–1944	Unknown	Poirier *et al.* (2000)
Monkey fetuses (*Erythrocebas patas*)	Brain, heart, kidney, liver, lung, placenta	In pregnant monkeys: 10 mg/day orally five days per week, last 9.5–10 weeks of gestation	7–246	Unknown	Olivero *et al.* (1997)
CD-1 mouse fetuses	Brain, kidneys, liver, lung, skin	In pregnant mice: 25 mg/day by gavage last seven days of gestation	7–101	Tumours of liver, lung and reproductive system	Olivero *et al.* (1997)

Table 2 (contd)

Sample source	Cell or tissue type	Dose of zidovudine or exposure	Molecules of zidovudine per 10^6 nucleotides	Mutagenic or carcinogenic response	Reference
Human cells	TK6 lympho-blastoid cells	In culture: 33 µmol/L for three days 100 µmol/L for three days 33 µmol/L plus 33 µmol/L for three days 100 µmol/L plus 100 µmol/L for three days		Significantly mutagenic at *TK* locus Significantly mutagenic at *TK* and *HPRT* loci Synergistic mutagenic effects at *TK* and *HPRT* loci Synergistic mutagenic effects at *TK* and *HPRT* loci	Meng *et al.* (2000b)
Human cells	TK6 lympho-blastoid cells	In culture: 300 µmol/L for three to six days	102 ± 15	Significantly mutagenic at *TK* locus	Sussman *et al.* (1999)

(*d*) *Genotoxicity of zidovudine in combinations*

In theory, a combination of two nucleoside analogues of two different bases could have at least an additive genotoxic effect in host cells. Meng *et al*. (2000b) tested two components of the frequently used clinical combination therapies, zidovudine and didanosine, by measuring the incorporation of zidovudine into nuclear DNA and the mutagenic responses at *HPRT* and *TK* in TK6 cells exposed to either drug alone and in combination at doses down to the peak plasma concentrations found in some patients. Didanosine was more cytotoxic than zidovudine, and didanosine alone was less mutagenic than zidovudine alone at both genes. An unexpected and striking finding was that equimolar combinations of zidovudine and didanosine not only significantly increased the *HPRT* and *TK* mutant frequencies at all doses, but the induced mutant frequencies at both loci were three to four times greater than the additive values obtained after exposure to zidovudine or didanosine alone. In addition, the amounts of zidovudine incorporated into DNA of cells exposed to zidovudine plus didanosine were 1.8 and 2.4 times greater than those in cells exposed to analogous concentrations of zidovudine alone. These results indicate that the mutagenic potentiation in human cells exposed to zidovudine plus didanosine is partly due to enhanced incorporation of zidovudine into cellular DNA. Further investigations are needed to determine if other clinically relevant combinations of antiretroviral agents have synergistic genotoxic effects in cell culture, in animal models and in human populations.

4.5 Mechanistic considerations

The toxicological and mutagenic consequences of exposure to zidovudine and the carcinogenic effects of this agent in rodents arise primarily as a result of its incorporation into host cell DNA and the concomitant termination of DNA replication. There is overwhelming evidence that zidovudine is incorporated into host cell nuclear and mitochondrial DNA in multiple systems, including cultured cells, animal models and humans. Incorporation values of about 7–500 molecules/10^6 nucleotides (approximately 10 000–100 000 zidovudine molecules/cell) have been obtained in adults and transplacentally exposed mice, monkeys (*E. patas* and *M. mulatta*) and humans given the drug alone or in therapeutic combinations (Table 2).

In human cells exposed to zidovudine, the amounts of zidovudine incorporated into cellular DNA correlated significantly with the frequencies of mutations induced in two target genes, strongly indicating that its incorporation into host cell DNA results in the observed mutagenicity. Consistent with the demonstrated chain termination, the potential for induction of large DNA deletions is supported by the consistent finding of clastogenicity (sister chromatid exchange, micronuclei and chromosomal aberrations) in human cells *in vitro* and in rodent cells *in vivo* after exposure to both high and low, clinically relevant doses. In addition, molecular analyses of mutations in zidovudine-exposed human cells have confirmed that the mutagenic response is mainly, but not

exclusively, due to the production of large deletions leading to loss of heterozygosity in autosomal cells.

The mutational spectra also suggest that zidovudine produces point mutations, either directly by some unknown mechanism or indirectly through inhibition of DNA polymerases, alterations in nucleotide pools, inhibition of DNA anabolic enzymes and/or interference with DNA repair processes. The presence of distinct activating point mutations in Ha-*ras* of zidovudine-induced mouse skin tumours suggests that multiple mechanisms, which include point mutations and clastogenic events, with concomitant loss of heterozygosity, are all likely to be involved in the process of zidovudine-induced tumour formation.

Although the phenomenon is not directly related to carcinogenesis, it is worth noting that zidovudine is also incorporated into mitochondrial DNA and inhibits mitochondrial DNA polymerases, with resultant effects on cell growth and viability. The amounts incorporated into mitochondrial DNA are similar to those in nuclear DNA, resulting in 1–10 molecules of zidovudine per mitochondrion.

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Zidovudine (AZT) is a nucleoside analogue that has been used in the treatment and prevention of HIV infection in adults and children since the mid-1980s. Zidovudine is in widespread use in combination regimens with other antiretroviral agents. It is currently indicated in the treatment of HIV-positive pregnant women and to prevent mother-to-infant transmission.

5.2 Human carcinogenicity data

No difference in the incidence of non-Hodgkin lymphoma relative to that in the general population was seen before and after introduction of zidovudine therapy.

In a large case–control study from the USA of HIV-infected patients, no association was found between the incidence of non-Hodgkin lymphoma and therapeutic use of zidovudine.

A large cohort study from the USA with limited length of follow-up suggested a linear increase in the cumulative risk for non-Hodgkin lymphoma over time among adult patients with AIDS, but this was not related to treatment with zidovudine. A number of other cohort studies were available which also involved limited length of follow-up and few subjects. No data were available on the risks for types of cancers other than non-Hodgkin lymphoma.

None of the studies provided information on the risk for cancer associated with use of zidovudine for more than three years.

5.3 Animal carcinogenicity data

Zidovudine was tested for carcinogenicity in mice and rats by oral administration, in mice by intravaginal administration and in mice by transplacental and by transplacental and postnatal exposure. Zidovudine was also administered with 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in a transplacental experiment in mice and in combination with α-interferon in mice.

Administration of zidovudine by gavage induced vaginal squamous-cell carcinomas in two studies in mice. A low incidence of vaginal tumours was observed in rats treated with the highest dose. Administration of zidovudine to mice by the intravaginal route resulted in an increased incidence of vaginal squamous-cell carcinomas. Combined administration of zidovudine with α-interferon also induced vaginal tumours in mice. Vaginal squamous-cell tumours are very rare in untreated animals.

Transplacental administration to mice resulted in an increased incidence and multiplicity of lung and liver tumours and in an increased incidence of female reproductive tract tumours in one study, whereas no increased tumour incidence was associated with treatment in another study at a lower dose. After transplacental and postnatal administration of zidovudine to mice, an increased incidence of vaginal squamous-cell carcinomas was seen. Zidovudine given transplacentally followed by postnatal topical application of TPA to mice resulted in an increased incidence and multiplicity of skin tumours (mostly papillomas).

5.4 Other relevant data

The pharmacokinetics of zidovudine in humans shows large inter- and intra-individual variation. The achievement of maximum plasma concentrations and removal from plasma of the parent compound are rapid except in patients with compromised renal function. The pharmacokinetics in nonhuman primates is virtually identical to that in humans. The absorption, distribution and elimination of zidovudine in rodents are more rapid than in humans, and its bioavailability is higher in rats and mice than in primates. Zidovudine is metabolized by three pathways: glucuronidation, which accounts for up to three-quarters of the human urinary product; mixed-function oxidase-mediated reactions, giving 3′-amino-3′-deoxythymidine, a minor urinary metabolite; and phosphorylation, which is fundamental to the antiviral activity of zidovudine but accounts for only about 1% of its total disposition. Unchanged zidovudine constitutes up to one-fifth of the human urinary products. In rats and mice, unchanged drug accounts for up to 90% of the urinary recovery, which represents about 80% of the dose; the remaining urinary products consist of five metabolites, which have been identified.

The serious adverse effects of treatment with zidovudine, reported in a small proportion of people, include haematotoxicity (anaemia, neutropenia), hepatotoxicity and cardiac and skeletal myopathy (due to mitochondrial effects). Similar toxic effects are found in treated mice.

Zidovudine crosses the placenta by bidirectional passive diffusion, and the drug and its monophosphate and monoglucuronide metabolites were observed in fetal tissue. Studies of children up to 4.2 years of age who had been exposed *in utero* and for up to six weeks after birth to zidovudine provided no evidence for an increased incidence of structural developmental abnormalities or cognitive or immune dysfunction. Studies in mice, rats and rabbits given zidovudine transplacentally showed no increase in the frequency of malformations, but some studies showed increased numbers of fetal resorptions and decreased fetal weights after oral administration of zidovudine at doses of 200–500 mg/kg bw per day during gestation. Studies in monkeys and rats indicated that the behavioural alterations in offspring exposed to zidovudine *in utero* were generally reversible.

Zidovudine is incorporated into nuclear and mitochondrial DNA in mammalian cells in culture, in experimental animals and in humans. It appears to cause mutations primarily by inducing large deletions, consistent with its action as a DNA chain terminator. It produces clastogenic effects in cultured human cells and in mice exposed to either high or clinically relevant concentrations. Analyses of mutations induced in human cells in culture and in skin tumours from transplacentally treated mice showed that exposure to zidovudine also causes point mutations.

5.5 Evaluation

There is *inadequate evidence* in humans for the carcinogenicity of zidovudine.

There is *sufficient evidence* in experimental animals for the carcinogenicity of zidovudine.

Overall evaluation

Zidovudine is *possibly carcinogenic to humans (Group 2B)*.

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